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ANTIBODIES

Field of the Invention

The present invention relates to antibodies that specifically bind to a C-terminal fragment of Apolipoprotein E (ApoE). The present invention also provides methods for obtaining such polypeptides and the use of such polypeptides in the diagnosis and treatment of Alzheimer's disease, systemic amyloidosis and other amyloid disorders.

Background to the Invention

Amyloidosis is a progressive, incurable metabolic disease of unknown cause characterized by abnormal deposits of protein in one or more organs or body systems. Amyloid proteins are manufactured, for example, by malfunctioning bone marrow. Amyloidosis, which occurs when accumulated amyloid deposits impair normal body function, can cause organ failure or death. It is a rare disease, occurring in about eight of every 1,000,000 people. It affects males and females equally and usually develops after the age of 40. At least 15 types of amyloidosis have been identified. Each one is associated with deposits of a different kind of protein.

The major forms of amyloidosis are primary systemic, secondary, and familial or hereditary amyloidosis. There is also another form of amyloidosis associated with Alzheimer's disease. Primary systemic amyloidosis usually develops between the ages of 50 and 60. With about 2,000 new cases diagnosed annually, primary systemic amyloidosis is the most common form of this disease in the United States. Also known as light-chain-related amyloidosis, it may also occur in association with multiple myeloma (bone marrow cancer). Secondary amyloidosis is a result of chronic infection or inflammatory disease. It is often associated with Familial Mediterranean fever (a bacterial infection characterized by chills, weakness, headache, and recurring fever), Granulomatous ileitis (inflammation of the small intestine), Hodgkin's disease, Leprosy, Osteomyelitis and Rheumatoid arthritis.

Familial or hereditary amyloidosis is the only inherited form of the disease. It occurs in members of most ethnic groups, and each family has a distinctive pattern of symptoms and organ involvement. Hereditary amyloidosis is though to be autosomal dominant, which means that only one copy of the defective gene is necessary to

cause the disease. A child of a parent with familial amyloidosis has a 50-50 chance of developing the disease.

Amyloidosis can involve any organ or system in the body. The heart, kidneys, gastrointestinal system, and nervous system are affected most often. Other common sites of amyloid accumulation include the brain, joints, liver, spleen, pancreas, respiratory system, and skin.

Alzheimer's disease (AD) is the most common form of dementia, a neurologic disease characterized by loss of mental ability severe enough to interfere with normal activities of daily living, lasting at least six months, and not present from birth. AD usually occurs in old age, and is marked by a decline in cognitive functions such as remembering, reasoning, and planning.

Between two and four million Americans have AD; that number is expected to grow to as many as 14 million by the middle of the 21st century as the population as a whole ages. While a small number of people in their 40s and 50s develop the disease, AD predominantly affects the elderly. AD affects about 3% of all people between ages 65 and 74, about 20% of those between 75 and 84, and about 50% of those over 85. Slightly more women than men are affected with AD, even when considering women tend to live longer, and so there is a higher proportion of women in the most affected age groups.

Several genes have been implicated in AD, including the gene for amyloid precursor protein, or APP, responsible for producing amyloid. Mutations in this gene are linked to some cases of the relatively uncommon early-onset forms of AD. Other cases of early-onset AD are caused by mutations in the presenilin genes, PS-1 and PS-2. A dementia similar to AD eventually affects nearly everyone with Downs syndrome, caused by an extra copy of chromosome 21. Other mutations on other chromosomes have been linked to other early-onset cases.

Potentially the most important genetic link was discovered in the early 1990s on chromosome 19. A gene on this chromosome, apoE, codes for a protein involved in transporting lipids into neurons.

Apolipoprotein E (ApoE) is a 34kDa glycosylated protein. The main sites of ApoE production are the liver and brain. ApoE is a constituent of very low density lipoprotein (VLDL), a subclass of high density lipoproteins and chylomicrons.

Cellular uptake of lipid complexes is mediated by binding of ApoE to the low density lipoprotein (LDL) receptor and other related receptors.

There are three major ApoE isoforms in humans, apoE2, apoE3 and apoE4 which are products of three alleles, ε2, ε3 and ε4. In the general population, the ε3 allele is the most common, accounting for 78% of all apoE alleles. The frequency of the ε4 allele is increased significantly in the population of late-onset sporadic and familial Alzheimer's disease (AD) patients.

ApoE contains a C-terminal domain (ApoE-CTD) and an N-terminal domain (ApoE-NTD) joined by a random-coil region. The C-terminal domain comprises a lipid binding site and the N-terminal domain binds to lipoprotein receptors. The CTD amino acid sequence is identical in all three isoforms of ApoE. The CTD and NTD may be separated by cleavage with thrombin.

Direct interactions between ApoE and Amyloid β (Aβ) have been demonstrated *in vitro*. ApoE is also present in AD plaques. It has been reported that the N-terminal domain of ApoE (ApoE-NTD) mediates binding of apoE to Aβ (Golabek *et al.*, (2000) Biophysical Journal 79: 1008-1015). However, AD plaques containing ApoE have been shown to comprise full-length ApoE at the centre of the plaques and a C-terminal domain fragment of ApoE (ApoE-CTD) at the periphery of the plaques (Cho *et al.*, (2001) J. Neuropathology and Expt. Neurology 60: 342-349). $A\beta_{1-42}$ deposition in plaques has been shown to precede ApoE deposition whilst $A\beta_{1-40}$ deposition follows ApoE depositon in plaque maturation (Terai *et al.*, (2001), Brain Research 900: 48-56).

The function of ApoE in the brain is not thought to be specific for AD. ApoE appears to play an important role in modifying recovery from acute brain injury. In particular, there is evidence from both clinical and animal studies to suggest that the presence of the ApoE4 isoform is associated with poor neurological recovery from a variety of acute brain injuries.

Summary of the Invention

The present inventors developed therapeutic antibodies directed to a region of Apolipoprotein E (ApoE) which is exposed in protein aggregates found in amyloid deposits including Alzheimer plaques but which is not accessible, or has only

restricted accessibility, in other forms of ApoE such as ApoE in lipoprotein particles in the blood.

Accordingly, the present invention provides:

- a human antibody or antibody fragment, which antibody or fragment:

(i) binds to a polypeptide having the amino acid sequence shown in SEQ ID NO: 1 of the C-terminal domain of Apolipoprotein E (ApoE-CTD) or the amino acid sequence of a part thereof; and

(ii) binds to human plaques;

- a human antibody or antibody fragment, which antibody or fragment:

(i) binds to a polypeptide having the amino acid sequence shown in SEQ ID NO: 1 of ApoE-CTD or the amino acid sequence of a part thereof; and

(ii) comprises a heavy chain CDR3 region comprising the sequence shown in SEQ ID NO: 20, SEQ ID NO: 512, SEQ ID NO: 513, SEQ ID NO: 514, SEQ ID NO: 515, SEQ ID NO: 516 or SEQ ID NO: 517;

- a human antibody or antibody fragment, which antibody or fragment:

(i) binds to a polypeptide having the amino acid sequence shown in SEQ ID NO: 1 of ApoE-CTD or the amino acid sequence of a part thereof; and

(ii) comprises a heavy chain CDR3 region comprising an amino acid sequence selected from the sequences shown in SEQ ID NO: 29, SEQ ID NO: 47, SEQ ID NO: 50, SEQ ID NO: 53, SEQ ID NO: 56, SEQ ID NO: 59, SEQ ID NO: 62, SEQ ID NO: 65, SEQ ID NO: 68, SEQ ID NO: 71, SEQ ID NO: 74, SEQ ID NO: 77, SEQ ID NO: 80, SEQ ID NO: 83, SEQ ID NO: 86 and SEQ ID NO: 89;

- a human antibody or antibody fragment, which antibody or fragment binds, in the presence of VLDL, to a polypeptide having the ApoE-CTD amino acid sequence shown in SEQ ID NO: 1 or the amino sequence of a part thereof;

- a human antibody or antibody fragment, which antibody or fragment:

(i) binds to human plaques; and

(ii) comprises a heavy chain CDR3 region comprising the sequence shown in SEQ ID NO: 20, SEQ ID NO: 512, SEQ ID NO: 513, SEQ ID NO: 514, SEQ ID NO: 515, SEQ ID NO: 516 or SEQ ID NO: 517;

- a human antibody or antibody fragment, which antibody or fragment:

(i) binds to human plaques; and

(ii) comprises a heavy chain CDR3 region comprising an amino acid sequence selected from the sequences shown in SEQ ID NO: 29, SEQ ID NO: 47, SEQ ID NO: 50, SEQ ID NO: 53, SEQ ID NO: 56, SEQ ID NO: 59, SEQ ID NO: 62, SEQ ID NO: 65, SEQ ID NO: 68, SEQ ID NO: 71, SEQ ID NO: 74, SEQ ID NO: 77, SEQ ID NO: 80, SEQ ID NO: 83, SEQ ID NO: 86 and SEQ ID NO: 89;

- an antibody or antibody fragment which comprises the heavy chain sequence shown in SEQ ID NO: 136 and the light chain sequence shown in SEQ ID NOS: 521 and 522;

- an antibody or antibody fragment which comprises the heavy chain sequence shown in SEQ ID NO: 142 and the light chain sequence shown in SEQ ID NO: 523;

- an antibody or antibody fragment which comprises the heavy chain sequence shown in SEQ ID NO: 40 and the light chain sequence shown in SEQ ID NO: 517 and/or 518;

- an antibody or antibody fragment which comprises the heavy chain sequence shown in SEQ ID NO: 40 and the light chain sequence shown in SEQ ID NO: 519 and/or 520;

- an antibody or antibody fragment which comprises the heavy chain CDR1 sequence shown in SEQ ID NO: 24, the heavy chain CDR2 sequence shown in SEQ ID NO: 25 and the heavy chain CDR3 sequence shown in any one of SEQ ID NOS: 207, 209 and 210;

- an antibody or antibody fragment which comprises the heavy chain CDR1 sequence shown in SEQ ID NO: 48, the heavy chain CDR2 sequence shown in SEQ ID NO: 49 and the heavy chain CDR3 sequence shown in any one of SEQ ID NOS: 320, 322 and 323;

- an antibody or antibody fragment which comprises the heavy chain CDR1 sequence shown in SEQ ID NO: 66, the heavy chain CDR2 sequence shown in SEQ ID NO: 67 and the heavy chain CDR3 sequence shown in SEQ ID NO: 373;

- an antibody or antibody fragment according to any one of the preceding claims which is a monoclonal antibody;

- an antibody or antibody fragment according to the invention, for use in a method of treatment of the human or animal body by therapy or in a diagnostic method practised on the human or animal body;

- use of an antibody or antibody fragment according to the invention, in the manufacture of a medicament for the treatment or prevention of an amyloid disorder;
- a pharmaceutical composition comprising an antibody or antibody fragment according to the invention and a pharmaceutically acceptable carrier or diluent;
- a method of treating a subject suffering from an amyloid disorder comprising administering to said subject a therapeutically effective amount of an antibody or antibody fragment according to the invention;
- a method of diagnosing an amyloid disorder in a subject comprising:

  (i) administering to said subject an antibody or antibody fragment according to the invention; and

  (ii) determining whether or not said antibody or antibody fragment binds to plaques in said subject, wherein binding of said antibody or antibody fragment to plaques is indicative of an amyloid disorder,

  thereby determining whether the subject has an amyloid disorder;
- a polynucleotide encoding an antibody or antibody fragment according to the invention;
- a vector comprising a polynucleotide according to the invention;
- a host cell expressing a polypeptide according the invention;
- a virus encoding a polynucleotide according to the invention;
- a kit for detecting ApoE-CTD, which kit comprises an antibody or antibody fragment according to the invention and means for detecting said an antibody or antibody fragment; and
- a method for detecting the presence of ApoE-CTD in a sample from a subject, which method comprises:

  (i) contacting a sample taken from a subject with an antibody or antibody fragment according to the invention under conditions that permit binding of the an antibody or antibody fragment to ApoE-CTD; and

  (ii) determining whether or not the an antibody or antibody fragment binds to the sample thereby detecting any ApoE-CTD present in the sample.

Brief Description of the Figures

Figure 1 shows the binding of known monoclonal antibodies to biotinylated CTD (bCTD) and VLDL in ELISA.

Figure 2 shows the binding of phage to bCTD (background = 0.05) and VLDL (background = 0.1).

Figure 3 shows an example of bCTD (A) and VLDL (B) ELISA on 203 phage clones.

Figure 4 shows Biacore affinity analysis on bCTD coated of sFab antibodies M27E11 (A), M28B02 (B) and M26F05 (C) and Biacore affinity analysis with Fab M27E11 coated on the chip (D).

Figure 5 is a schematic diagram showing the strategy of transfer of Fab to pBh1.

Figure 6 shows the binding of antibodies 807A-M0028-B02 (M28BO2) (A), 807A-M0026-F05 (M26F05) (B) and 807A-M0027-E11 (M27E11) (C) to human CTD, murine CTD and primate CTD.

Figure 7 shows the binding of control antibody PH1 (A) and 807A-M0028-B02 (M28B02) (B) to bCTD and bNTD.

Figure 8 shows the binding of control antibody PH1 (A) and 807A-M0028-B02 (M28B02) (B) to coated VLDL.

Figure 9 shows the results of antibody binding in competition ELISA between coated bCTD (0.05 μg/ml) and an excess of VLDL or CTD in solution.

Figure 10 shows the Biacore analysis of 807A-M0026-F05 (M26F05) as soluble Fab (top) and IgG (bottom) on a CTD-control chip.

Figure 11 shows the Biacore analysis of 807A-M0027-E11 (A) and 807A-M0028-B02 (B) as soluble Fab (top) and IgG (bottom) on a CTD-coated chip.

Figure 12 and 13 show the binding of bCTD in solution to Fab 807A-M0027-E11 (M27E11) indirectly coupled to a Biacore chip.

Figure 14 is a schematic diagram showing the strategy used to transfer V-regions from pBh1 to pRmk2a.

Figure 15 shows the sequences of the CTD peptides.

Figure 16 shows the selection campaign of Example 21.

Figure 17 shows the selection campaign of Example 22.

Figure 18 shows the selection campaign of Example 23.

Figure 19 shows a 'cut and paste' antibody reformatting strategy.

Figure 20 shows that 807A-M0028-B02 plaque binding in human AD brain sections is not blocked in presence of VLDL. Binding of 807A-M0028-B02 to human amyloid plaques *in vitro* was not affected by the presence of VLDL indicating that the antibody has very low affinity for CTD in VLDL relative to CTD in plaques.

Figure 21 shows the results of an *in vitro* immunohistochemistry (IHC) screen of sFab antibody clones on human AD brain sections. Several antibodies (Fab clones) from different selections were identified that bind to AD plaques by immunohistochemistry. Note that individual clones may have the same short name i.e. E11 but are from different selections and not identical. (C11= 807B-M0004-H03; (selection A) E11=807A-M0027-E11; B2=807A-M0028-B02; (selection B) E11= 807B-M0083-E11; D10= 807B-M0079D10; A3=807B-M0004-A03; A12= 807B-M0013-A12)

Figure 22 shows representative results (duplicate samples) of the concentration dependent binding of 807A-M0028-B02, 807A-M0028-B02.1 and 807A-M0028-B02.2 to human, primate and murine ApoE-CTD.

Figure 23 shows representative results (duplicate samples) of the concentration dependent binding of 807A-M0028-B02, 807A-M0028-B02.1, 807A-M0028-B02.2, 807B-M0004-H03, 807B-M0004-H03.1, 807B-M0004-A03 and 807B-M0004-A03.1 to human VLDL.

Figure 24 shows the detection of 807A-M0028-B02 plaque binding in brain tissue sections from APP/PS1 mouse:

a) *In vivo* binding of 807A-M0028-B02 4 days after injection;

b) *In vivo* binding of 807A-M0028-B02 7 days after injection.

Figure 25 shows results of a screen of *in vivo* plaque binding capacity of 807B M0004H03, 807B-M0004-A03, 807B-M0079-D10 and 807B-M0009-F06. Immunohistochemistry expression patterns of anti-CTD hIgG clones B807B-M0004H03, 807B-M0004-A03, 807B-M0079-D10 and 807B-M0009-F06 in APP/PS1 mouse brain sections after *in vivo* administration is shown.

Figure 26 shows results of an *in vitro* screen for plaque binding capacity of affinity maturated Fab-clones in human AD brain sections. Wild-type clone 807B-M0004-A03 (wt A03) was compared to affinity maturated clones 807B-M0118-B09 (BO9), 807B-M0117-F05 (F05), 807B-M0117-G01 (G01) and 807B-M0118-F03

(F03). Amyloid plaques visualised by anti-CTD binding antibodies (Fab clones) on human AD brain sections.

Figure 27 shows the strategy used for affinity maturation of 807A-M0028-B02, 807B-M0004-H03, 807B-M004-A03, 807B-M0079-D10 and 807B-M0009-F06.

Brief Description of the Sequences

SEQ ID NO: 1 is the amino acid sequence of the ApoE-CTD.

SEQ ID NO: 2 is the amino acid sequence of peptide 1 (amino acids 1 to 16 of the ApoE-CTD).

SEQ ID NO: 3 is the amino acid sequence of peptide 2 (amino acids 17 to 32 of the ApoE-CTD).

SEQ ID NO: 4 is the amino acid sequence of peptide 3 (amino acids 33 to 48 of the ApoE-CTD).

SEQ ID NO: 5 is the amino acid sequence of peptide 4 (amino acids 49 to 64 of the ApoE-CTD).

SEQ ID NO: 6 is the amino acid sequence of peptide 5 (amino acids 65 to 80 of the ApoE-CTD).

SEQ ID NO: 7 is the amino acid sequence of peptide 6 (amino acids 9 to 24 of the ApoE-CTD).

SEQ ID NO: 8 is the amino acid sequence of peptide 7 (amino acids 25 to 40 of the ApoE-CTD).

SEQ ID NO: 9 is the amino acid sequence of peptide 8 (amino acids 41 to 56 of the ApoE-CTD).

SEQ ID NO: 10 is the amino acid sequence of peptide 9 (amino acids 57 to 72 of the ApoE-CTD).

SEQ ID NO: 11 is the amino acid sequence of peptide 10 (amino acids 73 to 84 of the ApoE-CTD).

SEQ ID NO: 12 is the amino acid sequence of an epitope in peptide 4 (amino acids 53 to 60 of ApoE-CTD).

SEQ ID NO: 13 is the amino acid sequence of an epitope in peptides 4 and 9 (amino acids 57 to 64 of ApoE-CTD).

SEQ ID NO: 14 is the amino acid sequence of an epitope in peptide 9 (amino acids 61 to 68 of ApoE-CTD).

SEQ ID NO: 15 is the amino acid sequence of an epitope in peptides 1 and 6 (amino acids 9 to 16 of ApoE-CTD).

SEQ ID NO: 16 is the amino acid sequence of an epitope in peptides 4 and 8 (amino acids 49 to 56 of ApoE-CTD).

SEQ ID NO: 17 is the amino acid sequence of an epitope in peptides 3 and 8 (amino acids 41 to 48 of ApoE-CTD).

SEQ ID NO: 18 is the amino acid sequence of peptides 1 and 6 (amino acids 1 to 24 of ApoE-CTD).

SEQ ID NO: 19 is the amino acid sequence of peptides 8 and 9 (amino acids 41 to 72 of ApoE-CTD).

SEQ ID NO: 20 is the amino acid sequence of the consensus CDR3 sequence from antibodies 807A-M0027-Ell and 807A-M0028-B02.

SEQ ID NOS: 21 to 164 are described in Table 8.

SEQ ID NO: 165 is the amino acid sequence of human ApoE4.

SEQ ID NO: 166 is the amino acid sequence of human ApoE3.

SEQ ID NO: 167 is the amino acid sequence of human ApoE2.

SEQ ID NO: 168 is the amino acid sequence of the mature form of human ApoE4.

SEQ ID NO: 169 is the amino acid sequence of the mature form of human ApoE3.

SEQ ID NO: 170 is the amino acid sequence of the mature form of human ApoE2.

SEQ ID NOS: 171 to 206 are described in Table 8.

SEQ ID NOS: 207 to 511 are described in Tables 38 to 42.

SEQ ID NO: 512 is the consensus amino acid sequence of the CDR3 regions of affinity matured clones of 807A-M0028-B02.

SEQ ID NO: 513 is the consensus amino acid sequence of the CDR3 regions of affinity matured clones of 807B-M0004-A03.

SEQ ID NO: 514 is the consensus amino acid sequence of the CDR3 regions of affinity matured clones of 807B-M0004-H03.

SEQ ID NO: 515 is the consensus amino acid sequence of the CDR3 regions of affinity matured clones of 807B-M0009-F06.

SEQ ID NO: 516 is the consensus amino acid sequence of the CDR3 regions of selected affinity matured clones of 807A-M0028-B02.

SEQ ID NO: 517 is the consensus amino acid sequence of the CDR3 regions of selected affinity matured clones of 807B-M0004-A03.

SEQ ID NO: 518 to 527 are defined in Table 21.

Detailed Description of the Invention

A. Polypeptides

The present invention provides antibodies that bind to a region on Apolipoprotein E (ApoE) which is exposed in the protein aggregates found in amyloid deposits such as Alzheimer plaques, but which is not present or accessible in other forms of ApoE, such as in lipoprotein particles in the blood.

For the purposes of this invention, the term "antibody", unless specified to the contrary, includes antibody fragments.

Typically, an antibody of the invention binds the C-terminal domain of Apolipoprotein E (ApoE-CTD), i.e. is reactive with ApoE-CTD. An antibody of the invention does not bind to the N-terminal domain of Apolipoprotein E (ApoE-NTD). The antibody typically binds to the form of ApoE present in human plaques in preference to the form of ApoE present in VLDL. Generally, the form of ApoE present in human plaques is ApoE-CTD. An antibody of the invention preferably binds ApoE-CTD in the presence of very low density lipoprotein (VLDL). An antibody of the invention may be one that binds to an epitope in ApoE-CTD, which epitope is not present in ApoE associated with VLDL. For example, the epitope may be one which is one not accessible or exposed to the antibody when ApoE is associated with VLDL. The epitope to which the antibody binds may typically be hidden in full-length ApoE present in VLDL and so the affinity of the antibody for ApoE is substantially less than its affinity for ApoE-CTD. The epitope to which the antibody binds is present only in ApoE-CTD and not in ApoE-NTD and so the antibody is typically devoid of binding to ApoE-NTD. Any binding of the antibody to ApoE-NTD is generally non-specific binding of a substantially lower affinity than

the specific binding of the antibody to ApoE-CTD. A substantially lower affinity is generally at least a two fold, three fold, five fold, 10 fold, 50 fold or 100 fold lower affinity.

An antibody of the invention thus preferentially binds or specifically binds to ApoE-CTD. An antibody "preferentially binds" or "specifically binds" to ApoE-CTD when it binds with preferential or high affinity to ApoE-CTD but does not substantially bind, does not bind or binds with only low affinity to other polypeptides. A variety of protocols for binding, competitive binding or immuno-radiometric assays to determine the specific binding capability of an antibody are well known in the art (see for example Maddox *et al*, J. Exp. Med. 158, 1211-1226, 1993). Such immunoassays typically involve the formation of complexes between the specific protein and its antibody and the measurement of complex formation. Typically an antibody of the invention, is capable of binding to ApoE-CTD having the sequence shown in SEQ ID NO: 1 with an affinity constant of at least 10^7 M^{-1}, preferably at least 10^8 M^{-1}, 10^9 M^{-1} or 10^{10} M^{-1}. An antibody of the invention, is preferably capable of preferentially binding to ApoE-CTD with an affinity that is at least two-fold, 10-fold, 50-fold, 100-fold or greater than its affinity for binding to a non-specific polypeptide such as BSA, casein, VLDL, ApoE-NTD or ApoE present in VLDL.

An antibody which specifically binds to ApoE-CTD typically displays at least 2x background binding in an ELISA on immobilised ApoE-CTD but less than 2x background, typically 1x background, to control proteins such as ApoE-NTD or streptavidin.

An antibody of the invention generally binds to human plaques. The term "human plaques" is intended to cover any amyloid deposits comprising at least one protein having an amino acid sequence encoded by a human gene. Preferably the human plaque is present in or derived from human tissue. More preferably the human plaque is present in a sample that has been obtained from a human subject. The human subject may have an amyloid disorder, such as systemic amyloidosis or Alzheimer's disease. The sample may be taken from any tissue or organ containing amyloid plaques. Suitable tissues and organs include brain, tongue, intestines, skeletal muscle, smooth muscle, nerves, skin, ligaments, heart, liver, spleen and kidneys. Where the subject has Alzheimer's disease, the sample is generally a brain

section. The brain section is typically obtained post-mortem. Fibrils prepared from any such sample are also included within the term "human plaques".

The human plaque may be present in or derived form a non-human animal which is transgenic for one or more, for example two or three, human proteins, which human protein(s) is/are found in amyloid deposits. The human protein is preferably ApoE but may be amyloid precursor protein (APP) (typically comprising the Swedish mutation) or presenilin.

Binding to human plaques may be determined by any suitable method. For example in an IHC assay, binding of an antibody to human plaques can be said to occur when a positive blind scored IHC signal is obtained after staining with <20μg/ml antibody in two amyloid deposit samples primarily tested or if one sample is negative in the primary test, at least two out of three samples subsequently tested indicates that an antibody binds to human plaques. The samples are preferably derived from different individuals and sectioned from tissue samples with histologically verified amyloid deposits which are IHC positive for an amyloid marker such as Aβ.

The ability of an antibody to bind to human plaques may be determined *in vivo* using a mouse or other non-human animal model, such as a rodent or primate, of Alzheimer's disease or systemic amyloidosis.

In such an assay, binding of an antibody to plaques may be determined using IHC. The antibody may be labelled prior to being tested. Binding to the plaque may be defined as positive blind scored IHC staining of amyloid after injection of ≤ 1 mg antibody, in single or multiple doses, in at least two out of three mice tested. The signal is generally compared to the signal from stained anatomically, sex and age matched tissue from negative isotype matched control antibody injected mice.

The term "epitope" as used herein refers to that portion of a molecule that makes contact with a particular binding polypeptide. An epitope may be linear, comprising an essentially linear amino acid sequence from the antigen or conformational, comprising sequences that are separated by other sequences but come together structurally to form a binding site for the polypeptide.

The epitope in ApoE-CTD to which the antibody binds may appear on ApoE-CTD after cleavage from full-length ApoE. Alternatively the epitope may appear following the interaction of ApoE-CTD with amyloid plaques, for example as a

result of binding of ApoE-CTD to Aβ. Cleavage of ApoE and/or binding of ApoE-CTD to amyloid plaques may result in the exposure of new linear (peptide) epitopes and/or to the exposure or formation of new conformational epitopes. The epitope to which a polypeptide of the invention binds may be hidden in VLDL-associated ApoE due to the interaction of ApoE with other components of VLDL. The polypeptide may bind specifically to a complex formed between ApoE-CTD and Aβ.

The amino acid sequence of ApoE-CTD is shown in SEQ ID NO: 1. ApoE-CTD epitopes may thus be formed by a linear or conformational sequence within the sequence of ApoE-CTD as shown in SEQ ID NO: 1. An antibody that binds to ApoE-CTD typically bind to an ApoE-CTD polypeptide having the whole sequence shown in SEQ ID NO: 1 but may also bind to a part of the amino acid sequence of SEQ ID NO: 1 such as to a peptide having an amino acid sequence as shown in any one of SEQ ID NOS: 2 to 19. Preferably, the antibody binds to one or more of the peptides shown in SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. The part of ApoE-CTD to which the antibody binds is at least a three amino acid fragment of SEQ ID NO: 1, preferably at least a five, six, seven or eight amino acid fragment, more preferably a 10, 12 or 16 amino acid fragment.

The polypeptide (or peptide) to which the antibody binds may be a recombinant polypeptide. The polypeptide may be in solution or, more preferably, may be attached to a solid surface. For example, the polypeptide may be attached to beads, such as magnetic beads.

The polypeptide may be biotinylated. The biotin molecule conjugated to the peptide may be used to immobilize the polypeptide on a solid surface by coupling biotin to streptavidin on the solid surface.

An antibody of the present invention suitable for use in treating or preventing Alzheimer's disease and/or systemic amyloidosis typically tests positive in an *ex vivo* phagocyte assay. A positive phagocyte assay is defined as positive blind scored confocal microscopy detection of phagocytes that contain amyloid after co-culture on amyloid tissue after applying ≤20 μg/ml of the antibody in at least two out of three cultures tested. The signal is generally compared to the signal from identical co-cultures containing the same concentration of a negative control antibody. A positive

phagocyte assay generally also results in the degradation of amyloid, for example as shown by Western blot to be less than one third the density of the Aβ-based remaining after up to three days of co-culture, as compared to blots from identical co-cultures containing the same concentration of a negative-control antibody.

Antibodies and other peptides for therapeutic use are typically of high affinity, preferably having an affinity of <1 nM, for ApoE-CTD, to enable them to function optimally even at the low concentrations in the brain that will build up after systemic injection.

The term "antibody" refers to a protein comprising at least one, and preferably two, heavy chain variable regions (VH) and/or at least one, preferably two, light chain variable regions (VL). The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions (CDR)", interspersed with regions that are more conserved, termed "framework regions (FR)". The extent of the FR and CDRs has been precisely defined (see, Kabat, *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition,* U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Chothia *et al.* (1987) *J. Mol. Biol.* 196: 901-917, which are incorporated herein by reference in their entirety). Each VH and VL is composed of three CDRs and four FRs arranged from N-terminus to C-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The VH or VL chain of the antibody can further include all or part of a heavy or light chain constant region, to thereby form a heavy or light immunoglobulin chain respectively. In one embodiment, the antibody is a tetramer of two heavy and two light chains, wherein the heavy and light chains are interconnected by, for example, disulphide bonds. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. The light chain constant region is comprised of one domain, CL. The variable region of the heavy and light chains contains a binding domain that interacts with antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues and factors, including various cells of the immune system and the first component of the complement system. The term "antibody" includes intact immunoglobulins of types IgA, IgG, IgE, IgD, IgM and subtypes thereof. A preferred immunoglobulin is IgG.

As used herein the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognised human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu conatant region genes as well as a myriad of immunoglobulin variable region genes. Full-length immunoglobulin light chains (about 25 kD or 214 amino acids) are encoded by a variable region gene at the N-terminus (about 110 amino acids) and a kappa or lambda constant region at the C-terminus. Full-length immunoglobulin heavy chains (about 50 kD or 446 amino acids) are encoded by a variable region gene at the N-terminus (about 116 amino acids) and one of the other aforementioned constant region genes at the C-terminus, e.g. gamma (encoding about 330 amino acids).

An antibody fragment of the invention is typically an antigen-binding fragment. The term "antigen-binding fragment" refers to one or more fragments of a full-length antibody that are capable of specifically binding to ApoE-CTD. Examples of binding fragments include (i) a Fab fragment (a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment (a bivalent fragment comprising two Fab fragments linked by a disulphide bridge at the hinge region; (iii) a Fd fragment (consisting of the VH and CH1 domains); (iv) a Fv fragment (consisting of the VH and VL domains of a single arm of an antibody); (v) a dAb fragment (consisting of the VH domain); (vi) an isolated CDR; (vii) a single chain Fv (scFv) (consisting of the VH and VL domains of a single arm of an antibody joined by a synthetic linker using recombinant means such that the VH and VL domains pair to form a monovalent molecule); (viii) diabodies (consisting of two scFvs in which the VH and VL domains are joined such that they do not pair to form a monovalent molecule; the VH of each one of the scFv pairs with the VL domain of the other scFv to form a bivalent molecule); (ix) bi-specific antibodies (consisting of at least two antigen binding regions, each region binding a different epitope). Preferably, the antibody fragment is a Fab fragment or single-chain antibody (scFv).

The sequences of preferred CDR1 domains are shown in SEQ ID NOS: 21, 24, 27, 30, 33, 36, 45, 48, 51, 54, 57, 60, 63, 66, 69, 72, 75, 78, 81, 84, 87, 93, 111, 117 and 123. Other preferred CDR1 domains are variants of these sequences in which one or more amino acids within the sequence have been deleted or, more preferably, substituted. Other preferred CDR1 domains are variants of the sequences

shown in any one of SEQ ID NOS: 21, 24, 27, 30, 33, 36, 45, 48, 51, 54, 57, 60, 63, 66, 69, 72, 75, 78, 81, 84, 87, 93, 111, 117 and 123 in which one or more amino acid has been inserted. Preferably, a variant CDR1 domain comprises one or more, for example two, three, four or five substitutions, preferably conservative substitutions. Examples of such CDR1 variant sequences are the LV-CDR1 sequences identified in Tables 38, 39, 40, 41 and 42. Preferred CDR1 sequences include SEQ ID NOS: 33, 219, 226, 218, 326, 93, 325, 391 and 394.

The sequences of preferred CDR2 domains are shown in SEQ ID NOS: 22, 25, 28, 31, 34, 37, 46, 49, 52, 55, 58, 61, 64, 67, 70, 73, 76, 79, 82, 85, 88, 94, 112, 118 and 124. Other preferred CDR2 domains are variants of these sequences in which one or more amino acids within the sequence have been deleted or, more preferably, substituted. Other preferred CDR2 domains are variants of the sequences shown in one of SEQ ID NOS: 22, 25, 28, 31, 34, 37, 46, 49, 52, 55, 58, 61, 64, 67, 70, 73, 76, 79, 82, 85, 88, 94, 112, 118 and 124 in which one or more amino acid has been inserted. Preferably, a variant CDR2 domain comprises one or more, for example two, three, four or five substitutions, preferably conservative substitutions. Examples of such CDR2 variant sequences are the LV-CDR2 sequences identified in Tables 38, 39, 40, 41 and 42. Preferred CDR2 sequences include SEQ ID NOS: 382, 386, 333, 334, 34, 247 and 252.

The sequences of preferred CDR3 domains are shown in SEQ ID NOS: 23, 26, 29, 32, 35, 38, 47, 50, 53, 56, 59, 62, 65, 65, 68, 71, 74, 77, 80, 83, 86, 89, 95, 113, 119 and 125. Other preferred CDR3 domains are variants of these sequence in which one or more amino acids within the sequence have been deleted or, more preferably substituted. Other preferred CDR3 domains are variants of the sequences shown in one of SEQ ID NOS: 23, 26, 29, 32, 35, 38, 47, 50, 53, 56, 59, 62, 65, 65, 68, 71, 74, 77, 80, 83, 86, 89, 95, 113, 119 and 125 in which one or more amino acid has been inserted. Preferably, a variant CDR3 domain comprises one or more, for example two or three, conservative substitutions.

Conservative substitutions are shown in the following Table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other.

ALIPHATIC	Non-polar	G A P
		I L V
	Polar-uncharged	C S T M
		N Q
	Polar-charged	D E
		K R
AROMATIC		H F W Y

Examples of variant CDR3 sequences are the HV-CDR3 and LV-CDR3 sequences identified in Tables 38, 39, 40, 41 and 42. Preferred variant CDR3 sequences are shown in SEQ ID NOS: 207, 209, 210, 35, 269, 252, 34, 322, 323, 320, 341, 373 and 378.

Preferred antibodies comprise (i) the VH sequence shown in SEQ ID NO: 39, or a variant thereof, and the VL sequence shown in SEQ ID NO: 42, or a variant thereof; (ii) the VH sequence shown in SEQ ID NO: 40, or a variant thereof, and the VL sequence shown in SEQ ID NO: 43, or a variant thereof; (iii) the VH sequence shown in SEQ ID NO: 41, or a variant thereof, and the VL sequence shown in SEQ ID NO: 44, or a variant thereof.

Other preferred antibodies comprise a heavy chain sequence selected from the sequences shown in SEQ ID NOS: 317, 318, 319, 369, 370, 371, 372 and 397 and optionally a light chain sequence selected from SEQ ID NOS: 43, 295, 294, 304, 347, 348, 357, 362, 406 and 418. More preferred antibodies include antibodies having the following combinations of heavy and light chain sequences: SEQ ID NOS: 319 and 43, SEQ ID NOS: 318 and 295, SEQ ID NOS: 318 and 294, SEQ ID NOS: 317 and 304, SEQ ID NOS: 370 and 347, SEQ ID NOS: 370 and 348, SEQ ID NOS: 371 and 348, SEQ ID NOS: 372 and 348, SEQ ID NOS: 369 and 357, SEQ ID NOS: 370 and 362, SEQ ID NOS: 397 and 406. SEQ ID NOS: 397 and 418.

Variant antibodies may be obtained by any suitable method. Typically variants with improved binding characteristics are selected by affinity maturation.

In a preferred embodiment, the antibody is a recombinant or modified anti-ApoE-CTD antibody, e.g. a chimeric, humanised, deimmunised or an *in vitro*

generated antibody. The term "recombinant" or "modified" antibody as used herein is intended to include all antibodies that are prepared, expressed, created or isolated by recombinant means, such as (i) antibodies expressed using a recombinant expression vector transfected into a host cell; (ii) antibodies isolated from a recombinant, combinatorial antibody library; (iii) antibodies isolated from an animal (e.g. a mouse) that is transgenic for human immunoglobulin genes; or (iv) antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant antibodies include humanised, CDR grafted, chimeric, deimmunised, *in vitro* generated antibodies and may optionally include constant regions derived from human germline immunoglobulin sequences.

An antibody according to the invention is a human antibody. The antibody may be a chimeric antibody, a recombinant antibody, a humanised antibody, a monoclonal antibody or a polyclonal antibody. Preferably the antibody is monoclonal.

The antibody may be conjugated to a functional moiety such as a drug, detectable moiety or a solid support.

Also within the scope of the invention are compositions comprising two or more antibodies which bind different epitopes of ApoE-CTD. The antibodies in the composition may bind overlapping epitopes. Antibodies that bind overlapping epitopes competitively inhibit the binding of each other to ApoE-CTD.

The antibody is preferably monospecific, e.g. a monoclonal antibody, or antigen-binding fragment thereof. Bispecific and multivalent antibodies are also provided, which bispecific or multivalent antibodies bind to two or more different epitopes of ApoE-CTD.

An antibody of the invention may be joined to a binding moiety such as biotin. For example, an antibody, preferably an IgG, may be biotinylated by incubation with sulfosuccinimidyl-2-(biotinamido) ethyl-1,3-dithiopropionate. A biotinylated IgG preferably comprises from 1 to 5 such as 2, 3 or 4 biotin groups.

An antibody of the invention may be in substantially isolated form. They may be mixed with carriers or diluents which will not interfere with their intended use and still be regarded as substantially isolated. They may also be in a

substantially purified form, in which case they will generally comprise at least 90%, e.g. at least 95%, 98% or 99%, of the polypeptides or dry mass of the preparation.

B. Methods For Identifying Antibodies

The invention also provides a method for identifying an antibody according to the invention. The method typically comprises identifying an antibody that binds to a polypeptide having the amino acid sequence as shown in SEQ ID NO: 1 or the amino acid sequence of a part thereof and that binds to human plaques. Either or both binding assays may be carried out in the presence of VLDL. The methods generally comprise providing a display library and screening the library to identify a member that encodes an antibody that binds to ApoE-CTD or a fragment thereof and/or to human plaques, preferably in the presence of VLDL. A display library is a collection of entities; each entity includes an accessible antibody component and a recoverable component that encodes or identifies the antibody component. The antibody component can be of any length, e.g., from three amino acids to over 300 amino acids for example 30, 100 or 200 amino acids and is typically an antibody fragment, preferably a Fab fragment. In a selection, the antibody component of each member of the library is probed with ApoE-CTD and if the antibody component binds to ApoE-CTD or fragment thereof, the display library member is identified, typically by retention on a support. Display library members that bind ApoE-CTD may also typically tested for binding to ApoE-NTD (negative selection).

Retained display library members are recovered from the support and analysed. The analysis can include amplification and a subsequent selection under similar or dissimilar conditions. For example, positive and negative selections can be alternated. The analysis can also include determining the amino acid sequence of the antibody component and purification of the antibody component for detailed characterisation.

A variety of formats can be used for display libraries and any suitable format may be used in a method of the invention. Preferred formats are phage display and cell-based display such as yeast display.

In phage display, the candidate antibodies are typically covalently linked to bacteriophage coat protein. The linkage results from translation of a nucleic acid encoding the candidate antibodies fused to the coat protein. The linkage can include

a flexible peptide linker, a protease site, or an amino acid incorporated as a result of suppression of a stop codon. Phage display is described, for example, in Ladner *et al.*, U.S. Patent No. 5,223,409; Smith (1985) *Science* 228:1315-1317; WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; WO 93/01288; WO 92/01047; WO 92/02809; WO 90/09690; de Haard *et al.* (1999) *J. Biol. Chem.* 274:18218-30; Hoogenboom *et al.* (1998) *Immunotechnology* 4: 1-20; Hoogenboom *et al.* (2000) *Immunol. Today* 2:371-8; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J.* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrard *et al.* (1991) *Bio/Technology* 9:1373-1377; Rebar *et al.* (1996) *Methods Enzymol.* 267:129-49; Hoogenboom *et al.* (1991) *Nuc. Acids Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and Lee *et al.* (2003) Trends In Biotechnology 21: 45-52.

Phage display systems have been developed for filamentous phage (phage fl, fd and M13) as well as other bacteriophage (e.g., T7 Bacteriophage and lambdoid phages; see, e.g., Santini (1998) *J. Mol. Biol.* 282:125-135; Rosenberg *et al.* (1996) *Innovations 6:1-6;* Houshmet *et al.* (1999) *Anal. Biochem.* 268:363-370). The filamentous phage display systems typically use fusions to a minor coat protein, such as gene III protein, and gene VIII protein, a major coat protein, but fusions to other coat proteins such as gene VI protein, gene VII protein, gene IX protein, or domains thereof can also been used (see, e.g., WO 00/71694). In a preferred embodiment, the fusion is to a domain of the gene III protein, e.g., the anchor domain or "stump," (see, e.g., U.S. Patent No. 5,658,727 for a description of the gene III protein anchor domain).

The valency of the candidate polypeptides can also be controlled. Cloning of the sequence encoding the polypeptide component into the complete phage genome results in multivariant display since all replicates of the gene III protein are fused to the polypeptide component. For reduced valency, a phagemid system can be utilised. In this system, the nucleic acid encoding the polypeptide component fused to gene III is provided on a plasmid, typically of length less than 700 nucleotides. The plasmid includes a phage origin of replication so that the plasmid is incorporated into bacteriophage particles when bacterial cells bearing the plasmid are infected with

helper phage, e.g., M13K07. The helper phage provides an intact copy of gene III and other phage genes required for phage replication and assembly. The helper phage has a defective origin such that the helper phage genome is not efficiently incorporated into phage particles relative to the plasmid that has a wild type origin.

Bacteriophage displaying the candidate antibodies can be grown and harvested using standard phage preparatory methods, e.g., PEG precipitation from growth media.

After selection of individual display phages, the nucleic acid encoding the selected candidate antibodies can be obtained by infecting cells using the selected phages. Individual colonies or plaques can be picked, the nucleic acid isolated and sequenced.

In a screening procedure to obtain ApoE-CTD binders according to this invention, a display library is contacted with and allowed to bind a target ApoE-CTD molecule, usually immobilised on a solid support. Non-binders are separated from binders. In various ways, the bound phage are liberated from the ApoE-CTD, collected and amplified. Since the phage can be amplified through infection of bacterial cells, even a few binding phage are sufficient to reveal the gene sequence that encodes a binding entity. Using these techniques it is possible to recover a binding phage that is about 1 in 20 million in the population. One or more libraries, displaying 10-20 million or more potential binding polypeptides each, can be rapidly screened to find high-affinity ApoE-CTD binders. When the selection process works, the diversity of the population falls with each round until only good binders remain, i.e., the process converges. Typically, a phage display library will contain several closely related binders (10 to 50 binders out of 10 million). Indications of convergence include increased binding (measured by phage titers) and recovery of closely related sequences.

In a cell-display library the candidate antibodies are displayed on the surface of a cell, e.g., a eukaryotic or prokaryotic cell. Exemplary prokaryotic cells include *E. coli* cells, *B. subtilis* cells and spores (see, e.g., Lu *et al.* (1995) *Biotechnology* 13:366). Exemplary eukaryotic cells include yeast (e.g., *Saccharomyces cerevisiae, Schizosaccharmyces pombe, Hanseulla,* or *Pichia pastoris*). Yeast surface display is described in, for example, Boder and Wittrup (1997) *Nature Biotech.* 15:553-557. A yeast display system that may be used to display immunoglobulin proteins such as

Fab fragments, and yeast mating may be used to generate combinations of heavy and light chains.

Yeast display has clear advantages over phage display in the application of affinity maturation of anti-ApoE-CTD antibodies. The most important advantage is that FACS selection may be used to quantitatively sort each yeast cell for its antigen binding. It is also possible to perform normalised selection so that variations in display level can be corrected, thus avoiding selection on the basis of avidity. This is particularly important when using a multivalent target antigen.

The display library may be a ribosome display library. In a ribosome display library mRNA and the candidate antibody encoded by the RNA can be physically associated by stabilising ribosomes that are translating the mRNA and have the nascent polypeptide still attached. Typically, high divalent Mg^{2+} concentrations and low temperature are used. See, e.g., Mattheakis *at al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:9022 and Hanes *at al.* (2000) *Nature Biotech.* 18:1287-92; Hans *at al.* (2000) *Methods Enzymol.* 328-404-30 and Schaffitzel *et al.* (1999) *J. Immunol. Methods* 231:119-35.

Another display library format utilises peptide-nucleic acid fusions. Polypeptide-nucleic acid fusions can be generated by the *in vitro* translation of mRNA that include a covalently attached puromycin group, e.g., as described in Roberts and Szostak (1997) *Proc. Acad. Sci. USA* 94:12297-12302, and U.S. Patent No. 6,207,446. The mRNA can then be reverse transcribed into DNA and crosslinked to the polypeptide.

Another display format that may be used is a non-biological display in which the antibody component is attached to a non-nucleic acid tag that identifies the antibody. For example, the tag can be a chemical tag attached to a bead that displays the antibody or a radiofrequency tag (See, e.g., U.S. Patent No. 5,874,214).

A parental binding domain is selected to serve as a structural template for the candidate antibodies. The binding domain may be a naturally occurring or synthetic protein, or a region or domain of a protein such as an immunoglobulin. The parental binding domain may be selected based on knowledge of a known interaction between the parental binding domain and ApoE-CTD but, but this is not critical. In fact, it is not essential that the parental binding domain have any affinity for ApoE-CTD at all: its purpose is to provide a structure from which a library can be generated, which

library will include one or more candidate antibodies that bind specifically to ApoE-CTD.

The candidate antibodies may be Fab fragments, single chain Fv molecules (scFV), single domain antibodies, camelid antibodies and camelized antibodies.

In a preferred embodiment, the parental binding domain comprises an immunoglobulin domain with antigen-binding activity, such as scFv, Fab or IgG. A typical display library displays candidate polypeptides that include a VH domain and a VL domain. As in the case of the Fab and other formats, the displayed antibody can include a constant region as part of a light or heavy chain. In one embodiment, each chain includes one constant region, e.g. as in the case of a Fab. In other embodiments, additional constant regions are displayed.

Display libraries are particularly useful, for example for identifying human or "humanised" antibodies that recognise human antigens. The *in vitro* display selection process surmounts the inability of a normal human immune system to generate antibodies against self-antigens.

Antibody libraries can be constructed by a number of processes (see, e.g. de Haard *et al* (1999) *J. Biol. Chem.* 274:18218-30; Hoogenboom *et al* (1998) *Immunotechnology* 4:1-20, and Hoogenboom *et al* (2000) *Immunol. Today* 21:371-8). Further, elements of each process can be combined with those of other processes. The processes can be used such that variation is introduced into a single immunoglobulin domain (e.g. VH or VL) or into multiple immunoglobulin domains (e.g. VH and VL). The variation can be introduced into an immunoglobulin variable domain, e.g. in the region of one or more of CDR1, CDR2, CDR3, FR1, FR2, FR3 and FR4, referring to such regions of either and/or both of heavy and light chain variable domains. In one embodiment, variation is introduced into all three CDRs of a given variable domain. In another preferred embodiment, the variation is introduced into CDR1 and CDR2, e.g. of a heavy chain variable domain. Any combination is feasible.

In a preferred embodiment the parental domain comprises the CDR3 sequence shown in any one of SEQ ID NOS: 23, 26, 29, 32, 35, 38, 47, 50, 53, 56, 59, 62, 65, 68, 71, 74, 77, 80, 83, 86, 89, 95, 113, 119 and 125. Amino acid substitutions at one or both of positions 2 and 3 of SEQ ID NO: 23 or 26 are preferred variations in candidate antibodies. Examples of variant VH-CDR3

sequences generated by antibody spiking are identified in Tables 38, 39, 40, 41 and 42. Preferred CDR3 sequences are shown in SEQ ID NOS: 207, 209, 210, 320, 322, 323 and 373. Consensus sequences for preferred CDR3 sequences are shown in SEQ ID NOS: 512, 513, 514, 515, 516, 517 and 20.

Examples of variant VL-CDR3 sequences generated by light chain shuffling are also shown in Tables 38, 39, 40, 41 and 42. Preferred VL-CDR3 sequences are shown in SEQ ID NOS: 35, 269, 275, 268, 341 and 378.

The parental domain preferably also comprises the other components of the VH chain, and optionally a VL chain, or the other components of the VL chain, and optionally a VH chain.

A second preferred parental domain comprises a CDR1 and/or CDR2 domain with the sequence shown in any one of SEQ ID NOS: 21, 24, 27, 30, 33, 36, 45, 48, 51, 54, 57, 60, 63, 66, 69, 72, 75, 78, 81, 84, 87, 93, 111, 117 and 123 or in SEQ ID NOS: 22, 25, 28, 31, 34, 37, 46, 49, 52, 55, 58, 61, 64, 67, 70, 73, 76, 79, 82, 85, 88, 94, 112, 118 and 124. The candidate polypeptides may be generated by DNA shuffling the CDR1 and/or CDR2 domains. Examples of variant VL-CDR1 and VL-CDR2 sequences are identified in Tables 38, 39, 40, 41 and 42. Preferred CDR1 sequences are shown in SEQ ID NOS: 33, 219, 226, 218, 93, 325, 326, 391 and 394. Preferred CDR2 sequences are shown in SEQ ID NOS: 34, 247, 252, 333, 334, 382 and 386.

A third preferred parental domain comprises the VL sequence shown in any one of SEQ ID NOS: 42, 43, 44, 151, 157, 159 and 161. Candidate polypeptides are typically generated by DNA shuffling of the entire VL sequence. Examples of shuffled light chain sequences are identified in Tables 38, 39, 40, 41 and 42. Preferred shuffled light chain sequences are shown in SEQ ID NOS: 43, 295, 294, 304, 347, 348, 357, 362, 406 and 418.

In one process, antibody libraries are constructed by inserting diverse oligonucleotides that encode CDRs into the corresponding regions of the nucleic acid. The oligonucleotides can be synthesized using monomeric nucleotides or trinucleotides. For example, Knappik *et al* ((2000) *J. Mol. Biol.* 296:57-86) describes a method for constructing CDR encoding oligonucleotides using trinucleotide synthesis and a template with engineered restriction sites for accepting the oligonucleotides.

In another process, an animal, e.g. a rodent, is immunised with the ApoE-CTD. The animal is optionally boosted with the antigen to further stimulate the response. Then spleen cells are isolated from the animal, and nucleic acid encoding VH and/or VL domains is amplified and cloned for expression in the display library.

In yet another process, antibody libraries are constructed from nucleic acid amplified from naïve germline immunoglobulin genes. The amplified nucleic acid includes nucleic acid encoding the VH and/or VL domain. Sources of immunoglobulin-encoding nucleic acids are described below. Amplification can include PCR, e.g. with primers that anneal to the conserved constant region, or another amplification method.

Nucleic acid encoding immunoglobulin domains can be obtained from the immune cells of, e.g. a human, a primate, mouse, rabbit, camel or rodent. In one example, the cells are selected for a particular property. B cells at various stages of maturity can be selected. In another example, the B cells are naïve.

In one embodiment, fluorescent-activated cell sorting (FACS) is used to sort B cells that express surface-bound IgM, IgD or IgG molecules. Further, B cells expressing different isotypes of IgG can be isolated. In another preferred embodiment, the B or T cell is cultured *in vitro*. The cells can be stimulated *in vitro*, e.g. by culturing with feeder cells or by adding mitogens or other modulatory reagents, such as antibodies to CD40, CD40 ligand or CD20, phorbol myristate acetate, bacterial lipopolysaccharide, concanavalin A, phytohemagglutinin or pokeweed mitogen.

In still another embodiment, the cells are isolated from a subject that has an immunological disorder, e.g. systemic lupus erythematosus (SLE), rheumatoid arthritis, vasculitis, Sjogren syndrome, systemic sclerosis or anti-phospholipid syndrome. The subject can be a human or an animal, e.g. an animal model for the human disease, or an animal having an analogous disorder. In yet another embodiment, the cells are isolated from a transgenic non-human animal that includes a human immunoglobulin locus.

In one preferred embodiment, the cells have activated a program of somatic hypermutation. Cells can be stimulated to undergo somatic mutagenesis of immunoglobulin genes, for example, by treatment with anti-immunoglobulin, anti-

CD40 and anti-CD38 antibodies (see, e.g. Bergthorsdottir *et al* (2001) *J. Immunol.* 166:2228). In another embodiment, the cells are naïve.

The nucleic acid encoding an immunoglobulin variable domain can be isolated from a natural repertoire by the following exemplary method. First, RNA is isolated from the immune cell. Full length (i.e. capped) mRNAs are separated (e.g. by degrading uncapped RNAs with calf intestinal phosphatase). The cap is then removed with tobacco acid pyrophosphatase and reverse transcription is used to produce the cDNAs.

The reverse transcription of the first (antisense) strand can be done in any manner with any suitable primer. See, e.g. de Haard *et al* (1999) *J. Biol. Chem.* 274:18218-30. The primer binding region can be constant among different immunoglobulins, e.g. in order to reverse transcribe different isotypes of immunoglobulin. The primer binding region can also be specific to a particular isotype of immunoglobulin. Typically, the primer is specific for a region that is 3' to a sequence encoding at least one CDR. In another embodiment, poly-dT primers may be used (and may be preferred for the heavy-chain genes).

A synthetic sequence can be ligated to the 3' end of the reverse transcribed strand. The synthetic sequence can be used as a primer binding site for binding of the forward primer during PCR amplification after reverse transcription. The use of the synthetic sequence can obviate the need to use a pool of different forward primers to fully capture the available diversity.

The variable domain-encoding gene is then amplified, e.g. using one or more rounds. If multiple rounds are used, nested primers can be used for increased fidelity. The amplified nucleic acid is then cloned into a display library vector.

Any method for amplifying nucleic acid sequences may be used for amplification. Methods that maximise, and do not bias, diversity are preferred. Suitable techniques for nucleic acid amplification include the polymerase chain reaction (PCR), transcription-based methods that utilise RNA synthesis by RNA polymerases to amplify nucleic acid (Sarker *et al* (1989) *Science* 244:331-34), NASBA (US Patent Nos. 5,130,238; 5,409,818; and 5,554,517) which utilises cycles of transcription, reverse-transcription and RnaseH-based degradation to amplify a

DNA sample, rolling circle amplification (RCA; US Patent No. 6,143,495) and strand displacement amplification (SDA; US Patent No. 5,624,825).

After a first set of binding antibodies is identified, the sequence information can be used to design other libraries biased for members having additional desired properties, e.g., discrimination between ApoE-CTD and full-length ApoE, preferably VLDL-associated ApoE. Such techniques make it possible not only to screen a large number of potential binding antibodies but also make it practical to repeat the binding/elution cycles and to build secondary, biased libraries for screening analog-displaying packages that meet initial criteria. Using these techniques, a biased library may be screened to reveal members that bind tightly (i.e., with high affinity) under the screening conditions.

Thus, in one preferred embodiment, display library technology may be used in an iterative mode. A first display library is used to identify one or more antibodies that bind ApoE-CTD and/or human plaques. These identified antibodies are then varied using a mutagenesis method to form a second display library. Higher affinity polypeptides are then selected from the second library, e.g., by using higher stringency or more competitive binding and washing conditions.

In affinity maturation protocols, the variation is preferably generated by amino acid substitutions but may also result from deletion or addition of amino acids.

The amino acid substitutions may be those which are expected to alter the binding properties of the domain without significantly altering its structure, at least for most substitutions. It is preferred that the amino acid positions that are selected for variation (variable amino acid positions) will be surface amino acid positions, that is, positions in the amino acid sequence of the domains which, when the domain is in its most stable conformation, appear on the outer surface of the domain (i.e., the surface exposed to solution). Most preferably the amino acid positions to be varied will be adjacent or close together, so as to maximise the effect of substitutions. In addition, extra amino acids can be added into the structure of the parental binding domain.

In some implementations, the mutagenesis is targeted to regions known or likely to be at the binding interface. Mutagenesis can be directed to the CDR regions of the heavy or light chains as described herein. Further, mutagenesis can be directed to framework regions near or adjacent to the CDRs. Mutagenesis can also

be limited to one or a few of the CDRs, e.g., to make precise step-wise improvements.

Effective affinity maturation requires 4 components (i) the rediversification of lead antibody genes (ii) display on either phage or yeast (iii) affinity selection (iv) screening of clones for improved affinity.

Alignment of Fabs showing the required binding properties, for example using a BLAST algorithm (e.g. Karlin and Altschul (1993) PNAS USA 90: 5873-5787) may be used to identify conserved residues in the CDR domains. Sequence similarity amongst the CDR loops may allow a prediction of the direct involvement of any amino acid in antibody affinity or specificity.

For example, the VH-CDR3 loops of antibodies 807A-M0028-B02 and 807A-M0027-E11 (SEQ ID NOS: 23 and 26) show striking similarity and show consensus over 4/6 amino acids (SEQ ID NO: 20). This suggests that the VH-CDR3 plays a role in antibody affinity and specificity.

An optimal antibody mutagenesis strategy introduces a minimal number of mutations at functionally relevant positions. This is achieved by both targeted and non-targeted mutagenesis procedures. Non-targeted mutagenesis procedures include chain shuffling which introduces large block changes in antibodies by rediversifying the whole VL gene or the VH CDR1-2 fragment. Typically, the VH-CDR3 loop is left untouched, as it may make significant contributions to binding affinity and specificity. Examples of chain shuffling are described in the following documents: Marks *et al.*, (1992) Nature Biotech 10: 779-783, Schier *et al.*, (1996) J. Mol Biol. 255, 28-43, Park *et al.*, (2000) BBRC. 275. 553-557 and Chames *et al.*, (2002) J. Immunol. 1110-1118. Although chain shuffling is a well-validated technique (particularly for antibodies with a low starting affinity) a possible disadvantage is that by making such large block changes in the antibody molecule that there may be an increased chance of disrupting multiple favourable contacts. However this could be compensated by the loss of unfavourable contacts or the generation of new contacts.

Other exemplary non-targeted mutagenesis techniques include: error-prone PCR (Leung *et al.* (1989) *Technique* 1:11-15), recombination, DNA shuffling using random cleavage (Stemmer (1994) *Nature* 389-391; termed "nucleic acid shuffling"), RACHITT™ (Coco *at al.* (2001) *Nature Biotech.* 19:354), site-directed mutagenesis

(Zooler *et al.* (1987) *Nucl. Acids Res.* 10:6487-6504), cassette mutagenesis (Reidhaar-Olson (1991) *Methods Enzymol.* 208:564-586) and incorporation of degenerate oligonucleotides (Griffiths *at al.* (1994) EMBO *J.* 13:3245).

Targeted mutagenesis procedures include hot spot mutagenesis, parsimonious mutagenesis, saturation mutagenesis, domain randomisation and domain walking. CDR mutagenesis can be done in a stepwise manner that is target CDR1, select an optimised loop and subsequently target CDR2 etc. The single most naturally diverse loop is the VH-CDR3 and it is generally accepted that as this loop is situated centrally in the antibody combining site that this is a critical determinant of antibody specificity and affinity. This means that there is a strong case for specifically targeting this loop.

In one example of iterative selection, the methods described herein are used to first identify an antibody from a display library that binds ApoE-CTD with at least a minimal binding specificity for a target or a minimal activity, e.g., an equilibrium dissociation constant for binding of greater than 1 nM, 10 nM, or 100 nM, and which binds human plaques and or/which retains binding activity in the presence of VLDL-associated ApoE. The nucleic acid sequence encoding the initial identified antibody is used as a template nucleic acid for the introduction of variations, e.g., to identify a second polypeptide that has enhanced properties (e.g., binding affinity, kinetics, or stability) relative to the initial antibody.

Antibodies according to the present invention may be isolated using display technology, in a manner to identify ApoE-CTD binding antibodies exhibiting particular preselected properties of binding and release. According to this methodology, two solution conditions may be preselected, i.e., binding conditions and release conditions. The binding conditions are a set of solution conditions under which is desired that a discovered antibody will bind the target ApoE-CTD; the release conditions are a set of solution conditions under which it is desired that a discovered antibody will not bind the ApoE-CTD (i.e. will dissociate from ApoE-CTD). The two conditions may be selected to satisfy any criterion of the practitioner, such as ease of attaining the conditions, compatibility with other purification steps, lowered cost of switching between conditions compared to other affinity media, etc. Preferably, the two solution conditions are selected so as not to adversely affect the stability or activity of the target protein ApoE-CTD and so as to

differ significantly with respect to at least one solution parameter. For example, in conducting the screening for suitable binding peptides described herein, binders are selected that dissociated from the target in the presence of an ethylene glycol-containing buffer, or conditions of lowered pH (i.e. pH 2), or combinations of those conditions, which differ from the conditions employed for binding. Another parameter that can be advantageously varied is the concentration of a salt, for example NaC1, in the binding and elution buffers.

An antibody which binds to ApoE-CTD typically has a minimal binding specificity for ApoE-CTD, for example an equilibrium constant for binding of greater than 1nM, 10nM or 100nM.

Since a slow dissociation rate can be predictive of high affinity, particularly with respect to interactions between antibodies and their targets, methods of off-rate selection can be used to isolate antibodies with a desired kinetic dissociation rate (i.e., reduced) for a binding interaction to an ApoE-CTD.

To select for slow dissociating antibodies from a display library, the library is contacted to an immobilised target, preferably ApoE-CTD. The immobilised target is then washed with a first solution that removes non-specifically or weakly bound antibodies. Then the immobilised target is eluted with a second solution that includes a saturation amount of free target, i.e., replicates of the target that are not attached to the particle. The free target binds to antibodies that dissociate from the target. Rebinding is effectively prevented by the saturating amount of free target relative to the much lower concentration of immobilised target.

The second solution can have solution conditions that are substantially physiological or that are stringent. Typically, the solution conditions of the second solution are identical to the solution conditions of the first solution. Fractions of the second solution are collected in temporal order to distinguish early from late fractions. Later fractions include biomolecules that dissociate at a slower rate from the target than biomolecules in the early fractions.

Further, it is also possible to recover display library members that remain bound to the target even after extended incubation. These can either be dissociated using chaotropic conditions or can be amplified while attached to the target. For example, phage bound to the target can be contacted to bacterial cells.

The ApoE-CTD used in a method of the invention may be in any suitable

form. ApoE-CTD typically has the amino acid sequence set out in SEQ ID NO. 1 or the amino acid sequence of a fragment thereof. The fragment of ApoE-CTD is typically at least three amino acids in length, preferably at least five, six, seven or eight amino acids in length and more preferably at least 10, 12 or 16 amino acids in length. Examples of suitable fragments are set out in SEQ ID NOS: 2 to 19. Preferred fragments are those having a sequence shown in any one of SEQ ID NOs: 2, 5, 7, 9, 10, 12, 13, 14, 15, 16 and 17. One or more ApoE-CTD peptide may be used in a screening assay of the invention.

The ApoE-CTD polypeptides are generally produced by recombinant means. Urea-denatured ApoE-CTD which has been recombinantly or naturally produced may be used in a method of the invention. Candidate polypeptides may additionally or alternatively be screened for binding to CTD in a polymeric form (ApoE-CTD binds to fibrils). Binding to a complex of ApoE-CTD and Aβ may also be monitored.

ApoE-CTD may be cleaved from recombinant or naturally occurring ApoE, for example by the action of thrombin.

The ApoE-CTD polypeptide or peptide may be immobilised on a support. Typically immobilisation is achieved by tagging or biotinylating the polypeptide for capture onto a surface. For example, the ApoE-CTD may comprise an S-S biotin group for attachment to streptavadin (for example on streptavadin-coated magnetic beads). Alternatively the ApoE-CTD may comprise a cysteine residue for coupling to a BSA carrier for immobilisation (for example on plastic). In this way a "CTD-coated chip" may be produced. Binding of candidate polypeptide to a CTD-coated chip may be analysed by BIACORE analysis.

Display library members may also be screened for binding to human plaques.

The display library screening methods described herein preferably include a selection or screening process that discards display library members that bind to a non-target molecule. Examples of non-target molecules include: streptavidin and (ii) ApoE-NTD.

In one implementation, a so-called "negative selection" step is used to discriminate between the target and related non-target molecule and a related, but distinct non-target molecule. The display library or a pool thereof is contacted to the non-target molecule. Members of the sample that do not bind the non-target are

collected and used in subsequent selections for binding to the target molecule or even for subsequent negative selections. The negative selection step can be prior to or after selecting library members that bind to the target molecule.

In another implementation, a screening step is used. After display library members are isolated for binding to the target molecule, each isolated library member is tested for its ability to bind to a non-target molecule (e.g., a non-target listed above). For example, a high-throughput ELISA screen can be used to obtain this data. The ELISA screen can also be used to obtain quantitative data for binding of each library member to the target. The non-target and target binding data are compared (e.g. using a computer and software) to identify library members that specifically bind to the target MHC-peptide complex.

An antibody or antibody fragment of the invention may bind to ApoE in the presence of VLDL or other lipoprotein particles. An antibody of the invention typically binds to ApoE with a minimal binding specificity for ApoE-CTD, e.g. an equilibrium constant for binding of greater than 1 nM or 100 nM in the presence of VLDL. The VLDL may be present in any suitable form. For example human plasma may be added to the binding assay. Up to 50% human plasma may be added to the assay, for example up to 10%, up to 20%, up to 30% or up to 40% human plasma may be included.

The candidate polypeptides may also be screened for binding to ApoE-NTD. ApoE-NTD may be produced recombinantly or may be cleaved from recombinant or naturally occurring ApoE, for example by the action of thrombin.

In one embodiment, the candidate polypeptides may be screened for binding to astrocytes. It is preferred but not essential, that the selected polypeptides do not bind to astrocytes or bind with a much lower affinity to astrocytes than to ApoE-CTD, for example a two-fold, five-fold, 10-fold, 20-fold or 50-fold lower affinity.

After selecting candidate display library members that bind to ApoE-CTD, each candidate display library member may be further analysed, e.g. to further characterise its binding properties for the target. Each candidate display library member can be subjected to one or more secondary screening assays. The assay can be for a binding property, a catalytic property, a physiological property (e.g. cytotoxicity, renal clearance, immunogenicity), a structural property (e.g. stability, conformation, oligomerisation state) or another functional property. The same assay

can be used repeatedly, but with varying conditions, e.g. to determine pH, ionic or thermal sensitivities.

As appropriate, the assays can use the display library member directly, a recombinant antibody produced from the nucleic acid encoding a displayed antibody, or a synthetic antibody synthesised based on the sequence of a displayed antibody. The assays preferably comprise determining whether or not an antibody that binds ApoE-CTD also binds to human plaques, or whether it binds to ApoE-CTD in the presence of VLDL. Exemplary assays for binding properties include ELISA, homogeneous binding assays such as fluorescence resonance energy transfer (FRET) and alpha-screen, surface plasmon resonance (SPR), protein assays and cellular assays.

Antibodies encoded by a display library can also be screened for a binding property using an ELISA. For example, each candidate antibody that binds ApoE-CTD is brought into contact with a microtitre plate whose bottom surface has been coated with ApoE-CTD, VLDL or ApoE-NTD. The plate is washed with buffer to remove non-specifically bound polypeptides. Then the amount of the polypeptide bound to the plate is determined by probing the plate with an antibody that can recognise the polypeptide, e.g. a tag or constant portion of the polypeptide. The antibody is linked to an enzyme such as alkaline phosphatase, which produces a colorimetric product when appropriate substrates are provided. The polypeptide can be purified from cells or assayed in a display library format, e.g. as a fusion to a filamentous bacteriophage coat. In another version of the ELISA, each polypeptide of a diversity strand library is used to coat a different well of a microtitre plate. The ELISA then proceeds using a constant target molecule to query each well. A polypeptide specifically binds ApoE-CTD in an ELISA if it displays at least 2x background on ApoE-CTD but less than 1x background on negative control proteins such as ApoE-NTD or streptavidin.

A homogeneous binding assay is an assay in which the binding interaction of candidate antibody with a target can be analysed after all components of the assay are added without additional fluid manipulations being required. For example, fluorescence resonance energy transfer (FRET) can be used as a homogenous assay (see, for example, Lakowicz *et al*, US Patent No. 5,631,169). Another example of a

homogenous assay is Alpha Screen (Packard Bioscience, Meriden, Connecticut, USA).

The homogenous assays can be performed while the candidate polypeptide is attached to the display library vehicle, e.g. a bacteriophage.

The binding interaction of a molecule isolated from a display library and a target can be analysed using Surface Plasmon Resonance (SPR). SPR or Biomolecular Interaction Analysis (BIA) detects biospecific interactions in real time, without labelling any of the interactants. Changes in the mass at the binding surface (indicative of a binding event) of the BIA chip result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)). The changes in the refractivity generate a detectable signal, which are measured as an indication of real-time reactions between biological molecules. Methods for using SPR are described, for example, in Szabo *et al* (1995) *Curr. Opin. Struct. Biol.* 5:699-705 and on-line resources provided by BIAcore International AB (Uppsala, Sweden).

Information from SPR can be used to provide an accurate and quantitative measure of the equilibrium dissociation constant (K_D), and kinetic parameters, including K_{on} and K_{off}, for the binding of a biomolecule to a target. Such data can be used to compare different biomolecules. For example, proteins encoded by nucleic acid selected from a library of diversity strands can be compared to identify individuals that have high affinity for the target or that have a slow K_{off}. This information can also be used to develop structure-activity relationships (SAR). For example, the kinetic and equilibrium binding parameters of matured versions of a parent protein can be compared to the parameters of the parent protein. Variant amino acids at given positions can be identified that correlate with particular binding parameters, e.g. high affinity and slow K_{off}. This information can be combined with structural modelling (e.g. using homology modelling, energy minimisation or structure determination by crystallography or NMR). As a result, an understanding of the physical interaction between the protein and its target can be formulated and used to guide other design processes.

Antibodies identified from the display library can be immobilised on a solid support, for example, on a bead or an array. For a protein array, each of the polypeptides is immobilised at a unique address on a support. Typically, the address

is a two-dimensional address. Protein arrays are described below (see, e.g. "Diagnostics").

A candidate antibody identified as binding to ApoE-CTD can be screened by transforming vector nucleic acid sequences that encode the antibody into a host cell such that antibodies are produced within the cell, secreted from the cell, or attached to the cell surface. The cells can be screened for antibodies that bind to ApoE-CTD, for example by detecting a change in a cellular phenotype or a cell-mediated activity. For example, the activity may be cell or complement-mediated cytotoxicity.

In another embodiment, the library of cells is in the form of a cellular array. The cellular array can likewise be screened for any appropriate detectable activity.

C. *Producing an Antibody*

Standard recombinant nucleic acid methods can be used to express an antibody of the invention. Generally, a nucleic acid sequence encoding the antibody is cloned into a nucleic acid expression vector. Of course, if the antibody includes multiple polypeptide chains, each chain must be cloned into an expression vector, e.g. the same or different vectors, that are expressed in the same or different cells. If the antibody fragment is sufficiently small, i.e. has less than 50 amino acids, it can be synthesised using automated organic synthetic methods. Methods for producing antibodies are also provided below.

The expression vector for expressing the antibody ligand can include, in addition to the segment encoding the polypeptide ligand or fragment thereof, regulatory sequences, including for example, a promoter, operably linked to the nucleic acid(s) of interest. Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available for generating the recombinant constructs of the present invention. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene, La Jolla, California, USA); pTrc99A, pKK223-3, pKK233-3, pDR540, and pRIT5 (Pharmacia, Uppsala, Sweden). Eukaryotic: pWLneo, pSV2cat, pOG44, PXTI, pSG (Stratagene) pSVK3, pBPV, pMSG and pSVL (Pharmacia). One preferred class of preferred libraries is the display library, which is described below.

Methods well known to those skilled in the art can be used to construct vectors containing an antibody of the invention and appropriate transcriptional/ translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. See, for example, the techniques described in Sambrook & Russell, *Molecular Cloning: A Laboratory Manual*, 3rd Edition, Cold Spring Harbor Laboratory, NY (2001) and Ausubel *et al*, *Current Protocols in Molecular Biology* (Greene Publishing Associates and Wiley Interscience, NY (1989)). Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, mouse metallothionein-I and various art-known tissue specific promoters.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g. the ampicillin resistance gene of *E. coli* and *S. cerevisiae* auxotrophic markers (such as *URA3, LEI2, HIS3* and *TRPl* genes), and a promoter derived from a highly expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), a-factor, acid phosphatase or heat shock proteins, among others. The polynucleotide of the invention is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, a nucleic acid of the invention can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g. stabilisation or simplified purification of expressed recombinant product. Useful expression-vectors for bacteria are constructed by inserting a polynucleotide of the invention together with suitable translation initiation and termination signals, optionally in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli, Bacillus*

subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but non-limiting example, useful expression vectors for bacteria can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEMl (Promega, Madison, Wisconsin, USA).

The present invention further provides host cells containing the vectors of the present invention, wherein the nucleic acid has been introduced into the host cell using known transformation, transfection or infection methods. For example, the host cells can include members of a library constructed from the diversity strand. The host cell can be a eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected, for example by calcium phosphate transfection, DEAE, dextran mediated transfection or electroporation (Davis, L. *et al*, *Basic Methods in Molecular Biology* (1986)).

Any host/vector system can be used to identify one or more of the target elements of the present invention. These include, but are not limited to, eukaryotic hosts such as HeLa cells, CV-1 cells, COS cells, Sf9 cells and HEK293T cells as well as prokaryotic hosts such as *E. coli* and *B. subtilis*. The most preferred cells are those which do not normally express the particular reporter polypeptide or protein or which expresses the reporter polypeptide or protein at low natural level.

The host of the present invention may also be a yeast or other fungi. In yeast, a number of vectors containing constitutive or inducible promoters may be used. For a review see, *Current Protocols in Molecular Biology*, Vol, 2, Ed. Ausubel *et al*, Greene Publish. Assoc. & Wiley Interscience, Ch. 13 (1988); Grant *et al* (1987) "Expression and Secretion Vectors for Yeast", *Methods Enzymol.* 153:516-544 (1987); and *The Molecular Biology of the Yeast Saccharomyces*, Eds. Strathern *et al*, Cold Spring Harbor Press, Vols. I and II (1982).

The host of the invention may also be a prokaryotic cell such as *E. coli*, other enterobacteriaceae such as *Serratia marescans,* bacilli, various pseudomonads or other prokaryotes which can be transformed, transfected and/or infected.

The present invention further provides host cells genetically engineered to contain the antibodies of the invention. For example, such host cells may contain nucleic acids of the invention introduced into the host cell using known transformation, transfection or infection methods. The present invention still further provides host cells genetically engineered to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the antibodies in the cell.

The host cell can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell.

Introduction of the recombinant construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran mediated transfection or electroporation (David, L. *et al*, (1986) *Basic Methods in Molecular Biology*). The host cells containing one of the polynucleotides of the invention can be used in a conventional manner to produce the gene product encoded by the isolated fragment (in the case of an ORF).

Any on suitable host/vector system can be used to express one or more of the diversity antibodies of the present invention. Various mammalian cell culture systems can also be employed to express recombinant antibodies.

Antibodies, e.g. Fabs, can be produced in bacterial cells, e.g. *E. coli* cells. For example, if the Fab is encoded by sequences in a phage display vector that includes a suppressible stop codon between the display entity and a bacteriophage protein (or fragment thereof), the vector nucleic acid can be shuffled into a bacterial cell that cannot suppress a stop codon. In this case, the Fab is not fused to the gene III protein and is secreted into the media.

Antibodies can also be produced in eukaryotic cells. In one embodiment, the antibodies (e.g. scFvs) are expressed in a yeast cell such as *Pichia* (see, e.g. Powers *et al* (2001) *J. Immunol. Methods.* 251:123-35), *Hanseula* or *Saccharomyces*.

In one preferred embodiment, antibodies are produced in mammalian cells. Preferred mammalian host cells for expressing the clone antibodies or antigen-binding fragments thereof include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin ((1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220), used with a DHFR selectable marker, e.g. as described in Kaufman and Sharp ((1982) *Mol. Biol.* 159:601-621), lymphocytic cell lines, e.g. NS0 myeloma cells and SP2 cells, COS cells and a cell from a transgenic animal, e.g. a transgenic mammal. For example, the cell is a mammary epithelial cell.

In addition to the nucleic acid sequence encoding the diversified immunoglobulin domain, the recombinant expression vectors may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g. origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, e.g. US Patent Nos. 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in *dhfr-* host cells with methotrexate selection/amplification) and the *neo* gene (for G418 selection).

In an exemplary system for recombinant expression of an antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the antibody heavy and the antibody light chain is introduced into *dhfr*$^-$ CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (e.g. derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carried a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector,

transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. For example, some antibodies can be isolated by affinity chromatography with a Protein A or Protein G.

For antibodies that include an Fc domain, the antibody production system preferably synthesises antibodies in which the Fc region is glycosylated. For example, the Fc domain of IgG molecule is glycosylated at asparagine 297 in the CH2 domain. This asparagine is the site for modification with biantennary-type oligosaccharides. It has been demonstrated that this glycosylation is required for effector functions mediated by Fc receptors and complement Clq (Burton and Woof (1992) *Adv. Immunol.* 51:1-84; Jefferis *et al* (1998) *Immunol. Rev.* 163:59-76). In one preferred embodiment, the Fc domain is produced in a mammalian expression system that appropriately glycosylates the residue corresponding to asparagine 297. The Fc domain can also include other eukaryotic post-translational modifications.

Antibodies can also be produced by a transgenic animal. For example, US Patent No. 5,849,992 describes a method of expressing an antibody in the mammary gland of a transgenic mammal. A transgene is constructed that includes a milk-specific promoter and nucleic acids encoding the antibody of interest and a signal sequence for secretion. The milk produced by females of such transgenic mammals includes, secreted-therein, the antibody of interest. The antibody can be purified from the milk, or for some applications, used directly.

An ApoE-CTD antibody of the invention may be isolated from the display library and its sequence and/or structure may be analysed. The antibody may be produced in any desired quantity using known methods. For example, the antibody may advantageously be produced by a chemical synthesis followed by treatment under oxidising conditions appropriate to obtain the native conformation, i.e., the correct disulfide bond linkages. Synthesis may be carried out by methodologies well known to those skilled in the art (see, Kelley *et al.*, in *Genetic Engineering Principles and Methods*, (Setlow, J.K., ed.), Plenum Press, NY., (1990) vol. 12, pp. 1-19; Stewart *et al.*, *Solid-Phase Peptide Synthesis* (1989), W.H. Freeman Co., San Francisco). Polypeptides according to the invention may also be prepared commercially by companies providing polypeptide synthesis as a service (e.g., BACHEM Bioscience, Inc., King of Prussia, Pa.; Quality Controlled Biochemicals, Inc., Hopkinton, Mass).

D. Diagnostic Methods

Antibodies that bind to ApoE-CTD and identified by the methods described herein and/or detailed herein have *in vitro* and *in vivo* diagnostic, therapeutic and prophylactic utilities.

In one aspect, the present invention provides a diagnostic method for detecting the presence ApoE-CTD *in vitro* (e.g., a biological sample, such as a biopsy or *in vivo* (e.g., *in vivo* imaging in a subject).

The method includes: (i) contacting a sample with an antibody of the invention; and (ii) detecting formation of a complex between the antibody and the sample. The method can also include contacting a reference sample (e.g., a control sample) with the antibody, and determining the extent of formation of the complex between the antibody and the sample relative to the same for the reference sample. A change, e.g., a statistically significant change, in the formation of the complex in the sample or subject relative to the control sample or subject can be indicative of the presence of ApoE-CTD in the sample.

Another method includes: (i) administering an antibody of the invention to a subject; and (ii) detecting formation of a complex between the antibody and the subject. The detection step can include determining location or time of formation of the complex.

The antibody ligand can be directly or indirectly labelled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials.

Complex formation between an antibody of the invention and ApoE-CTD can be detected by measuring or visualising either the antibody bound to the ApoE-CTD or unbound antibody. Conventional detection assays can be used, e.g., an enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay (RIA) or tissue immunohistochemistry. Further to labelling the antibody, the presence of ApoE-CTD can be assayed in a sample by a competition immunoassay utilising standards labelled with a detectable substance and an unlabelled antibody. In one example of this assay, the biological sample, the labelled standards and the antibody are combined and the amount of labelled standard bound to the unlabeled ligand is

determined. The amount of ApoE-CTD in the sample is inversely proportional to the amount of labelled standard bound to antibody.

Fluorophore and chromophore labelled antibodies can be prepared. Since antibodies absorb light having wavelengths up to about 310 nm, the fluorescent moieties should be selected to have substantial absorption at wavelengths above 310 nm and preferably above 400 nm. A variety of suitable fluoresces and chromophores are described by Stryer (1968) *Science* 162:526 and Brand, L. *at al.* (1972) *Annual Review of Biochemistry* 41:843-868. The antibodies can be labelled with fluorescent chromophore groups by conventional procedures such as those disclosed in U.S. Patent Nos. 3,940,475, 4,289,747 and 4,376,110. One group of fluorescers having a number of the desirable properties described above is the xanthene dyes, which include the fluoresceins and rhodamines. Another group of fluorescent compounds are the naphylamines. Once labelled with a fluorophore or chromophore, the antibody can be used to detect the presence or localisation of the ApoE-CTD in a sample, e.g., using fluorescent microscopy (such as confocal or deconvolution microscopy).

Immunohistochemistry can be performed using the antibodies described herein. For example, the antibody can synthesised with a label (such as a purification or epitope tag), or can be detectably labelled, e.g., by conjugating a label or label-binding group. For example, a chelator can be attached to the antibody. The antibody is then contacted to a histological preparation, e.g., a fixed section of tissue that is on a microscope slide. After an incubation for binding, the preparation is washed to remove unbound antibody. The preparation is then analysed, e.g., using microscopy, to identify if the antibody bound to the preparation.

Of course, the antibody can be unlabelled at the time of binding. After binding and washing, the antibody is labelled in order to render it detectable.

The antibody can also be immobilised on a protein array. The protein array can be used as a diagnostic tool, e.g., to screen medical samples (such as isolated cells, blood, sera, biopsies, and the like). Of course, the protein array can also include other ligands, e.g., that bind to the ApoE-CTD.

Methods of producing polypeptide arrays are described, e.g., in De Wildt *et al.* (2000) *Nature Biotech.* 18:989-994; Lueking *et al.* (1999) *Anal. Biochem.* 270:103-111; Ge (2000) *Nuc. Acids Res.* 28:e3; MacBeath and Schreiber (2000)

Science 289:1760-1763; WO 01/40803 and WO 99/51773A1. Polypeptides for the array can be spotted at high speed, e.g., using commercially available robotic apparati, e.g., from Genetic MicroSystems and Affymetrix (Santa Clara, California, USA) or BioRobotics (Cambridge, UK). The array substrate can be, for example, nitrocellulose, plastic, glass, e.g., surface-modified glass. The array can also include a porous matrix, e.g., acrylamide, agarose or another polymer.

For example, the array can be an array of antibodies, e.g., as described in De Wildt, *supra*. Cells that produce the polypeptide ligands can be grown on a filter in an arrayed format. Polypeptide production is induced, and the expressed polypeptides are immobilised to the filter at the location of the cell.

An antibody array can be contacted with a labelled target to determine the extent of binding of the target to each immobilised antibody from the diversity strand library. If the target is unlabeled, a sandwich method can be used, e.g., using a labelled probed, to detect binding of the unlabeled target.

Information about the extent of binding at each address of the array can be stored as a profile, e.g., in a computer database. The antibody array can be produced in replicates and used to compare binding profiles, e.g., of a target and a non-target. Thus, antibody arrays can be used to identify individual members of the diversity strand library that have desired binding properties with respect to one or more molecules.

In still another embodiment, the invention provides a method for detecting the presence of a ApoE-CTD containing plaque *in vivo*. The method includes (i) administering to a subject (e.g., a patient having Alzheimer's disease or systemic amyloidosis) an antibody of the invention, conjugated to a detectable marker; (ii) exposing the subject to a means for detecting said detectable marker bound to the ApoE-CTD containing plaque. For example, the subject is imaged, e.g., by NMR or other tomographic means.

Examples of labels useful for diagnostic imaging in accordance with the present invention include radiolabels such as ^{131}I, ^{111}In, ^{123}I, ^{99m}Tc, ^{32}P, ^{125}I, ^{3}H, ^{14}C, and ^{188}Rh, fluorescent labels such as fluorescein and rhodamine, nuclear magnetic resonance active labels, positron emitting isotopes detectable by a positron emission tomography ("PET") scanner, chemiluminescers such as luciferin and enzymatic markers such as peroxidase or phosphatase. Short-range radiation emitters, such as

isotopes detectable by short-range detector probes can also be employed. The polypeptide ligand can be labelled with such reagents using known techniques. Foe example, see Wensel and Meares (1983) *Radioimmunoimaging and Radioimmunotherapy*, Elsevier, New York for techniques relating to the radiolabel of antibodies and D. Colcher *et al.* (1986) *Methods Enzymol.* 121:802-816.

A radiolabel ligand of this invention can also be used for *in vitro* diagnostic tests. The specific activity of a isotopically-labelled ligand depends upon the half-life, the isotopic purity of the radioactive label, and how the label is incorporated into antibody.

Procedures for labelling polypeptides with the radioactive isotopes (such as ^{14}C, ^{3}H, ^{35}S, ^{125}I, ^{32}P, ^{131}I) are generally known. For example, tritium labelling procedures are described in U.S. Patent No. 4,302,438. Iodinating, tritium labelling, and ^{35}S labelling procedures, e.g., as adapted for murine monoclonal antibodies, are described, e.g., by Goding, J.W. (*Monoclonal Antibodies: Principles And Practice: Production And Application Of Monoclonal Antibodies In Cell Biology, Biochemistry, And Immunology* 2nd ed., London, Orlando, Academic Press (1986) polypeptide. 124-126) and the references cited therein. Other procedures for iodinating polypeptides, such as antibodies, are described by Hunter and Greenwood (1962) *Nature* 144:945, David *et al.* (1974) *Biochemistry* 13:1014-1021, and U.S. Patent Nos. 3,867,517 and 4,376,110. Radiolabelling elements which are useful in imaging include ^{123}I, ^{131}I, ^{111}In, and ^{99m}Tc, for example. Procedures for iodinating antibodies are described by Greenwood, F. *et al.* (1963) *Biochem. J.* 89:114-123; Marchalonis, J. (1969) *Biochem. J.* 113:299-305; and Morrison, M. *et al.* (1971) *Immunochemistry* 8:289-297. Procedures for ^{99m}Tc-labeling are described by Rhodes, B. *et al.* in Burchiel, S. *et al.* (eds.), *Tumor Imaging: The Radioimmunichemical Detection of Cancer,* New York: Masson 111-123 (1982) and the references cited therein. Procedures suitable for ^{111}In-labeling antibodies are described by Hnatowich, D.J. *et al.* (1983) *J. Immun. Methods* 65:147-157, Hnatowich, D. *et al.* (1984) *J. Applied Radiation* 35:554-557 and Buckley, R.G. *et al.* (1984) *F.E.B.S. Lett.* 66:202-204.

In the case of a radiolabelled antibody, the antibody is administered to the patient, is localised to the plaque with which the antibody reacts, and is detected or "imaged" *in vivo* using known techniques such as radionuclear scanning using e.g., a

gamma camera or emission tomography. Alternatively, a position emission transaxial tomography scanner, such as designated Pet VI located at Brookhaven National Laboratory, can be used where the radiolabel emits positrons (e.g., ^{11}C, ^{18}F, ^{15}O, and ^{13}N).

Magnetic Resonance Imaging (MRI) uses NMR to visualise internal features of a living subject, and is useful for prognosis, diagnosis, treatment, and surgery. MRI can be used without radioactive tracer compounds for obvious benefit. Some MRI techniques are summarised in published European patent application EP-A-0 502 814. Generally, the differences related to relaxation time constants T1 and T2 of water protons in different environments is used to generate an image. However, these differences can be insufficient to provide sharp high resolution images.

The differences in these relation time constants can be enhanced by contrast agents. Examples of such contrast agents include a number of magnetic agents paramagnetic agents (which primarily alter T1) and ferromagnetic or superaramagnetic (which primarily alter T2 response). Chelates (e.g., EDTA, DTPA and NTS chelates) can be used to attach (and reduce toxicity) of some paramagnetic substances (e.g., Fe^{+3}, Mn^{+2}, Gd^{+3}). Other agents can be in the form of particles, e.g., less than 10 μm to about 10 nM in diameter). Particles can have ferromagnetic, antiferromagnetic or superparamagnetic properties. Particles can include, e.g., magnetic (Fe_3O_4), $-Fe_2O_3$, ferrites and other magnetic mineral compounds of transition elements. Magnetic particles may include one or more magnetic crystals with and without nonmagnetic material. The nonmagnetic material can include synthetic or natural polymers such as sepharose, dextran, dextrin, starch and the like.

Antibodies of the invention can also be labelled with an indicating group containing of the NMR-active ^{19}F atom, or a plurality of such atoms inasmuch as (i) substantially all of naturally abundant fluorine atoms are the ^{19}F isotope and, thus, substantially all fluorine-containing compounds are NMR-active; (ii) any chemically active polyfluorinated compounds such as trifluoracetic anhydride are commercially available at relatively low cost, and (iii) many fluorinated compounds have been found medically acceptable for use in humans such as the perfluorinated polyethers utilised to carry oxygen as hemoglobin replacements. After permitting such time for incubation, a MRI scan is carried out using an apparatus such as one of those described by Pykett (1982) *Scientific American* 246:78-88.

Also within the scope of the invention are kits comprising an antibody of the invention and instructions for diagnostic use, e.g., the use of the antibody to detect ApoE-CTD, *in vitro*, e.g., in a sample, e.g., a biopsy from a patient having systemic amyloidosis, or *in vivo*, e.g., by imaging a subject. The kit can further contain a least one additional reagent, such as a label or additional diagnostic agent. For *in vivo* use the antibody can be formulated as a pharmaceutical composition.

E. Therapeutic Methods

Polypeptides that bind to ApoE-CTD and identified by the methods described herein and/or detailed herein have therapeutic and prophylactic utilities. For example, these ligands can be administered to cells in culture, e.g. *in vitro* or *ex vivo*, or in a subject, e.g. *in vivo*, to treat, prevent and/or diagnose a variety of disorders such as Alzheimer's disease or systemic amyloidosis.

As used herein, the term "treat" or "treatment" is defined as the application or administration of an anti-ApoE-CTD antibody, alone or in combination with, a second agent to a subject, e.g. a patient, who has a disorder (e.g. a disorder as described herein), a symptom of a disorder or a predisposition toward a disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder.

As used herein, an amount of an anti-ApoE-CTD polypeptide effective to treat a disorder, or a "therapeutically effective amount" refers to an amount of the ligand which is effective, upon single or multiple dose administration to a subject, or in prolonging curing, alleviating, relieving or improving a subject with a disorder as described herein beyond that expected in the absence of such treatment.

As used herein, an amount of an anti-ApoE-CTD polypeptide effective to prevent a disorder, or a "prophylactically effective amount" of the polypeptide refers to an amount of an anti-ApoE-CTD polypeptide, e.g. an anti-ApoE-CTD antibody described herein, which is effective, upon single- or multiple-dose administration to the subject, in preventing or delaying the occurrence of the onset or recurrence of a disorder, e.g. Alzheimer's disease.

The terms “induce”, “inhibit”, “potentiate”, “elevate”, “increase”, “decrease” or the like, e.g. which denote quantitative differences between two states, refer to a difference, e.g. a statistically significant difference, between the two states.

As used herein, the term “subject” is intended to include human and non-human animals. Preferred human animals include a human patient having a disorder characterised by abnormal cell proliferation or cell differentiation. The term “non-human animals” of the invention includes all vertebrates, e.g. non-mammals (such as chickens, amphibians, reptiles) and mammals, such as non-human primates, sheep, dog, cow, pig, etc.

The term “amyloid disorders” is intended to include, but not limited to, Alzheimer’s disease, primary systemic amyloidosis, secondary systemic amyloidosis, senile systemic amyloidosis, familial amyloid polyneuropathy I, familial amyloid polyneuropathy III, familial non-neuropathic amyloidosis, hereditary cerebral amyloid angiopathy, Familial British Dementia (FBD), Haemodialysis-related amyloidosis, Familial amyloidosis (Finnish type), Familial subepithelial corneal amyloid, Type II diabetes, Hereditary renal amyloidosis, Pituitary-gland amyloidosis, Injection localized amyloidosis, Medullary carcinoma of the thyroid, Atrial amyloidosis, Familial Danish Dementia (FDD), and Downs syndrome. Related to amyloid diseases wherein amyloid fibrils are detected, comprise, but is not limited to, Spongiform encephalopathies, Sporadic Creutzfeldt-Jakob disease, Familial Creutzfeldt-Jakob disease, Iatropic prion disorders, Variant Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker Disease (GSS), Kuru, Parkinson′s disease, Huntington’s disease, Familial amyotrophic lateral sclerosis (ALS), and Chronic obstructive pulmonary disease.

Furthermore, amyloid conditions can be defined as disorders with amyloid deposits in brain, medulla or other organs. An example of such disorders is Alzheimer’s disease. Other dementia disorders characterized by amyloid deposits are Spongiform encephalopathies, Sporadic Creutzfeldt-Jakob disease, Familial Creutzfeldt-Jakob disease, Iatropic prion disorders, Variant Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker Disease (GSS), Kuru, Parkinson′s disease, Huntington’s disease, Familial British Dementia, Familial Danish Dementia, Down syndrome, Primary Systemic amyloidosis, such as Immunoglobulin-light-chain-related amyloidosis, Secondary Systemic amyloidosis, such as Amyloidosis related

to amyloid A protein, Familial systemic amyloidosis, such as Familial transthyretin-associated amyloidosis, Familial apolipoprotein A-I associated amyloidosis, Familial gelsolin associated amyloidosis, Familial fibrinogen A α associated amyloidosis, Familial lyzosome amyloidosis, Senile Systemic amyloidosis, Familial amyloid polyneuropathy I, Familial amyloid polyneuropathy III, Familial non-neuropathic amyloidosis, Hereditary cerebral amyloid angiopathy, Haemodialysis-related amyloidosis, Familial amyloidosis, finnish type, Familial subepithelial corneal amyloid, Type II diabetes, Hereditary renal amyloidosis, Pituitary-gland amyloidosis, Injection localized amyloidosis, Medullary carcinoma of the thyroid, Atrial amyloidosis, Chronic obstructive pulmonary disease, and Familial amyotrophic lateral sclerosis-ALS. Detailed references can be found in James C. Sacchettini and Jeffery W. Kelly: Nature Reviews, Drug Discovery, Vol. 1 April 2002, 267-275.

In one embodiment, the subject is a human subject. Alternatively, the subject can be a mammal expressing an ApoE-CTD-like antigen with which a polypeptide of the invention cross-reacts. A polypeptide of the invention can be administered to a human subject for therapeutic purposes (discussed further below). Moreover, an anti-ApoE-CTD polypeptide can be administered to a non-human mammal expressing the ApoE-CTD-like antigen to which the polypeptide binds (e.g. a primate, pig or mouse) for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of the polypeptide (e.g. testing of dosages and time courses of administration).

For *in vivo* embodiments, the contacting step is effected in a subject and includes administering the anti-ApoE-CTD polypeptide to the subject under conditions effective to permit both binding of the ligand to the plaque and the treating, e.g. the destruction of the plaque.

Methods of administering anti-ApoE-CTD polypeptides are described in "Pharmaceutical Compositions". Suitable dosages of the molecules used will depend on the age and weight of the subject and the particular drug used.

The anti-ApoE-CTD ligands can be used directly *in vivo* to eliminate ApoE-CTD-containing plaques via natural complement-dependent cytoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC). The polypeptides of the invention can include complement binding effector domain, such as the Fc portions from IgG1,

-2, or -3 or corresponding portions of IgM which bind complement. The treatment can be supplemented by the addition of complement or serum containing complement. Further, phagocytosis of plaques coated with a polypeptide of the invention can be improved by binding of complement proteins.

Antibody-targeted amyloid plaques can be internalised by microglia through type A scavenger receptors (Melanie I. Brazil, Haeyong Chung, and Frederick R. Maxfield. *Effects of Incorporation of Immunoglobulin G and Complement Component C1q on Uptake and Degradation of Alzheimer's Disease Amyloid Fibrils by Microglia* J. Biol. Chem., May 2000; 275: 16941-16947). Alternatively, other mechanisms independent of the microglial Fc receptor might play a role in clearing diffuse, 3D6-immunoreactive, Thio-S-negative plaques and soluble Aß moieties (Wilcock DM, DiCarlo G, Henderson D, Jackson J, Clarke K, Ugen KE, Gordon MN, Morgan D: *Intracranially administered anti-Abeta antibodies reduce beta-amyloid deposition by mechanisms both independent of and associated with microglial activation.* J Neurosci 2003, 23:3745-3751). Consistent with this hypothesis, Fc-knockout mice also showed reduction of plaque burden after Aß immunotherapy (Das P, Howard V, Loosbrock N, Dickson D, Murphy MP, Golde TE: *Amyloid-beta immunization effectively reduces amyloid deposition in FcRgamma–/– knock-out mice.* J Neurosci 2003, 23:8532-8).

Also encompassed by the present invention is a method of killing or ablating which involves using the anti-ApoE-CTD ligand for prophylaxis. For example, these materials can be used to prevent or delay development or progression Alzheimer's disease, systemic amyloidosis or other amyloid disorders.

Use of the therapeutic methods of the present invention to treat Alzheimer's disease or systemic amyloidosis has a number of benefits. Since the polypeptides specifically recognise ApoE-CTD, other tissue is spared and high levels of the agent are delivered directly to the site where therapy is required. Treatment in accordance with the present invention can be effectively monitored with clinical parameters. Alternatively, these parameters can be used to indicate when such treatment should be employed.

F. Pharmaceutical Compositions

In another aspect, the present invention provides compositions, e.g. pharmaceutically acceptable compositions, which include an antibody of the invention formulated together with a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutical compositions" encompasses labelled ligands for *in vivo* imaging as well as therapeutic compositions.

As used herein, "pharmaceutically acceptable carrier" includes any physiologically compatible solvents, dispersion media, coatings, and the like. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g. by injection or infusion). Depending on the route of administration, the active compound, i.e. polypeptide may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-sold and solid dosage forms, such as liquid solutions (e.g. injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for administration of humans with antibodies. The preferred mode of administration is parental (e.g. intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the anti-ApoE-CTD polypeptide is administered by intravenous infusion or injection. In another preferred embodiment, the anti-ApoE-CTD ligand is administered by intramuscular or subcutaneous injection.

The phrases "parenteral administration" and administered parentally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnod, intraspinal, epidural and intrasternal injection and infusion.

Pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage.

The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (i.e. the polypeptide) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilisation. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

The antibodies of the present invention can be administered by a variety of methods known in the art, although for many applications, the preferred route/mode of administration is intravenous injection or infusion. For example, for therapeutic applications, the antibody can be administered by intravenous infusion at a rate of less than 30, 20, 10, 5 or 1 mg/min to reach a dose of about 1 to 100 mg/m^2 such as 7 to 25 mg/m^2. The route and/or mode of administration will vary depending upon the desired results.

Pharmaceutical compositions can be administered with medical devices known in the art. For example, in a preferred embodiment, a pharmaceutical composition of the invention can be administered with a needleless hypodermic injection device, such as the devices disclosed in US Patent Nos. 5,399,163, 5,383,851, 5,312,335, 5,064,413, 4,941,880, 4,790,824 or 4,596,556. Examples of well-known implants and modules useful in the present invention include: US Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a precise infusion rate; US Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; US Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-

chamber compartments; and US Patent No. 4,475,196, which discloses an osmotic drug delivery system. Of course, many other such implants, delivery systems and modules are also known.

In certain embodiments, the compounds of the invention can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB, they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g. US Patent Nos. 4,522,811, 5,374,548 and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhancing targeted drug delivery (see, e.g. V.V. Ranade (1989) *J. Clin. Pharmacol.* 29:685).

Dosage regimens are adjusted to provide the optimum desired response (e.g. a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parental compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody of the invention is 0.1-20 mg/kg, more preferably 1-10 mg/kg. The antibody can be administered by intravenous infusion at a rate of less than 30, 20, 10, 5 or 1 mg/min to reach a dose of about 1 to 100 mg/m^2 or about 5 to 30 mg/m^2. For antibody fragments which have lower molecular weights than an IgG, appropriate amounts can be proportionally less. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be

further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody of the invention. The desired therapeutic result is typically a lessening or amelioration of one or more symptom of the disease or disorder from which the individual being treated is suffering. A therapeutic amount of an antibody of the invention may be an amount which serves to slow down or stop production of amyloid deposits, eliminate existing amyloid deposits, alleviate underlying disorders (that give rise to secondary amyloidosis), and relieve symptoms caused by heart or kidney damage. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the composition may vary according to factors such as the disease state, age, sex and weight of the individual, and the ability of the polypeptide ligand to elicit a desired response in the individual. A therapeutically effective amount is also one is which any toxic or detrimental effects of the composition is outweighed by the therapeutically beneficial effects. A "therapeutically effective dosage" preferably inhibits a measurable parameter, e.g. plaque formation or growth rate by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit a measurable parameter can be evaluated in an animal model system predictive of efficacy in humans. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit such inhibition *in vitro* by assays known to the skilled practitioner.

A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. The desired prophylatic result is the inhibition or delay in the outset or progression of symptoms associated with the disease it is intended to prevent in the individual being treated. Typically, since a prophylactic dose is used in subject prior to or at an

earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Also within the scope of the invention are kits comprising an antibody of the invention and instructions for use, e.g. treatment, prophylactic or diagnostic use. In one embodiment, the instructions for diagnostic applications include the use of the antibody to detect the form ApoE-CTD associated with plaques, *in vitro*, e.g. in a sample, e.g. a biopsy or cells from a patient having Alzheimer's disease or systemic amyloidosis, or *in vivo*. In another embodiment, the instructions for therapeutic applications include suggested dosages and/or modes of administration in a patient with Alzheimer's disease or systemic amyloidosis. The kit can further contain at least one additional reagent, such as diagnostic or therapeutic agent.

The following invention is further illustrated by the following Examples, which should not be construed as further limiting. The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference in their entirety.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

Examples

Example 1: Antibody Library composition

Antibodies that bind to ApoE found in plaques of amyloid disorders that do not bind to VLDL were selected from human phage antibodies in the Dyax phagemid library Fab300. Antibody diversity is present in the library used for the selections on CTD, the diversity in the light and heavy chains are composed as follows:

Heavy chains

The heavy chain consists of one heavy chain gene segment (V3-23, or DP-47), in which diversity is created using synthetic oligonucleotides in certain positions in the HCDR1 and HCDR2. The distribution pattern is based on a diversity analysis

of natural sequences. The appended HCDR3 diversity is derived from natural occurring sequences of the IgM-pool of B-cells from a series of autoimmune donors.

Light chains

The light chain repertoire is derived from a pool of naturally rearranged light chains sequences, from the same source as the H-CDR3 diversity. This means that we can expect Vkappa and Vlambda genes, based on a diverse range of germline segments and with somatic mutations in or outside the CDRs.

Control Antibodies

The following anti-ApoE antibodies were used as controls:

3D12, a mouse antibody that binds to CTD, VLDL, LDL, ApoE2, ApoE3 and ApoE4 (Colabek *et al.* Biophysical J., 79:1008-1015);

E19, a goat antibody directed to CTD (Weisgraber (1986), J. Biol.Chem., 261: 2068-2076); and

6C5, a mouse antibody directed to NTD (Castano 1995) J. Biol. Chem., 270: 17610 – 17615.

Example 2: Preparation and pretesting of fibrils

Fibrils were extracted from spleen and kidney. Insoluble amyloid fibrils were extracted from human tissues by repeated rounds of mechanical homogenisation in cold 0.15M NaC1, 0.1% NaN3, with subsequent centrifugation in order to rescue the amyloid in the pellet. Finally the amyloid was dissolved/suspended in water and stored. Amyloid content was verified by Congo red staining of suspension smears. This method of extracting fibrils is known in the art, see for example Skinner *et al.* Prep. Biochem. 1982;12(5): 461-476. It was determined that bound and non-bound phage could be separated by washing with 5 Marvel-PBS-Tween (0.1%) washes, 2 PBS-Tween washes and 1 PBS wash.

Example 3: Preparation and pretesting of biotinylated CTD (bCTD):

CTD having the amino acid sequence shown in SEQ ID NO: 1 was used. Biotinylation was performed with sulfo-NHS-SS-biotin according to the method described by Pierce using molar ratios of CTD/biotin of 1/2 and 1/10. SDS PAGE

revealed that 100% of the material was labelled. In mass-spectrometry at a ratio of 1/10, three to five of the five possible biotinylation sites were labelled with biotin. At a ratio of 1/2 bCTD showed one or two biotins per molecule which is favourable for keeping the structure of the molecule. All CTD and NTD used were labelled with the same protocol at a ratio 1/2 and tested in SDS-page.

Coated bCTD was prepared by coating BSA with biotin, washing, adding streptavidin and, after washing again, adding b-CTD.

To produce denatured bCTD, bCTD was treated with urea and, after washing, bound to streptavidin on biotinylated BSA. To be sure that the bCTD binds and stays on the beads during urea treatment, we measured the amount of bCTD before and after urea treatment and binding to the beads. No loss of bCTD was seen.

Example 4: Selections on fibrils and ur-bCTD

In the selection strategy the aim was to select for antibodies binding to the complex form of CTD in amyloid fibrils. A first round of selection was performed on fibrils from kidney, a second round of selection was performed on fibrils from spleen and rounds three and four of selection were performed on ur-bCTD. No enrichment was found in rounds two and three but in round four there was an enrichment of 1379. In a second strategy, three rounds of selections on bCTD were performed.

A pre-screening was then carried out to decide which round of selection to chose for a high throughput screen and which antigen should be used. There was no significant difference seen between screening on bCTD and ur-bCTD. It was, therefore, decided to carry out a high throughput screen on the ur-bCTD selection with streptavidin coated bCTD. As a negative control bBSA, streptavidin coated plates were used.

Example 5: Sequence and binding analysis

Screening of more than 2000 clones was performed on coated bCTD. ELISA was performed in an automated system. The same method was used to screen for NTD binders. Clones, binding to bCTD and not binding to bNTD were analysed further.

Sequencing was carried out for heavy and light chains genes for clones positive in the high throughput bCTD phage ELISA. Dyax's proprietary 'Webphage' software was used to analyse the sequences of clones.

752 clones were screened after round 3 (23 positives) and 216 clones after round 4 (216 positives) of the selections on fibrils and ur-bCTD. 940 clones were screened after round 2 of selections on bCTD. The number of positive clones in ELISA using bCTD was 463. The number of correct sequences obtained was 163.

To analyse the sequence data, comparisons of sequences at different levels were carried out, including the following:

(1) overall diversity = number of different sequences, will identify a clone as different if one amino acid difference is found (dubbed VH+VL diversity) = 163

(2) heavy chain diversity = number of clones with a different heavy chain, ignoring the light chain sequence = 152

(3) HCDR3 diversity = the number of clones with a different heavy chain CDR3 = 54 (+ 2 clones with amber HCDR3).

This three-level analysis was carried out for two reasons:

Firstly, it is generally accepted that the heavy chain, and, in that domain, its HCDR3 region, are of major importance for the epitope recognition of the antibody. All antibodies with an identical H-CDR3 sequence were grouped.

Secondly, the design of the diversity of the library is such that antibody variants that have an identical HCDR3 with mutations in the other CDRs of the heavy chain, and sometimes identical, sometimes different light chains are expected.

Example 6: Binding of antibodies to bCTD and VLDL

To monitor binding to VLDL, VLDL was coated on a microtiter plate, and incubated with the test antibodies. A secondary antibody-HRP detection method is used to detect bound antibodies. Staining is performed with tetramethylbenzidine (TMB) and H_2O_2. Only non-bound antibody is washed away. Binding to VLDL will give a high signal in ELISA.

As a positive control we used monoclonal antibodies against ApoE. Two antibodies (3D12, E19), binding to CTD and VLDL, were positive in bCTD ELISA (Figure 1A) and in VLDL ELISA (Figure 1B). Another antibody (6C5) that binds to NTD does not bind to bCTD but binds well to VLDL. This NTD site is not covered

by VLDL and could give a measure of coating quality of ApoE itself. Since of this antibody signal is high, we can conclude that enough ApoE is coated to perform the VLDL ELISA like it is.

For phage antibodies (Figure 2), we made a classification in 3 groups: antibodies that are always positive (more than 3 times of negative phage binding), antibodies that are sometimes positive, sometimes negative (sometimes 2 times negative phage binding, sometimes negative) and negative antibodies. For the doubtful antibodies the ELISA is possibly not sensitive enough, or the antibodies are just not binding or probably the affinity is not high enough to see a high signal or there could be a cross-reaction with VLDL (epitope partially on VLDL, partially on covered CTD) etc.

Example 7: VLDL Assay development and Automation

The VLDL ELISA was performed for all 203 bCTD positive phage clones. 6 clones were found which were always positive (more than 3 times the background). Other clones produced a signal higher than 2 times the background (Figure 3). These clones are not excluded from further testing at this stage. Only 6 clones were positive, with a high signal, to VLDL. Tests were carried out 3 times, and the same results were obtained. In parallel binding of antibodies to bNTD was tested. No antibodies bound to bNTD. Clones that bound to VLDL were not tested further.

Example 8: Recloning of phage to Fab, specificity tests

Because of the low amount of VLDL binders in coated VLDL ELISA and because of the variable results of the VLDL competition ELISA, we batch recloned in parallel all different 157 clones from Fab on phage into soluble Fab. It was expected that many Fabs will not bind to bCTD because of their monovalent nature versus the multivalent phage, thus enabling low affinity binders to be excluded by means of Fab ELISA signals on bCTD.

After recloning, 85 antibodies bound specifically to bCTD. No new VLDL or NTD binders were found. The amino acid sequences of CDR regions of the VH and VL chains of these antibodies are shown in Tables 9 and 10.

Example 9: Epitope mapping

Binding to identical epitopes is tested by monitoring competition between Fab and phage antibodies. A limited amount of phage and a maximal amount of Fab is added to an ELISA well coated with bCTD. After binding steps, phage is detected by a peroxidase reaction after incubating with an anti-M13 HRP antibody. Because of the high concentration of Fab added, phage directed to the same epitope as the Fab will be competed off and the Fab signal will be decreased.

Antibodies from the same VH-CDR3 group recognise overlapping epitopes. This criterion was used to exclude clones for immunohistochemistry (IHC): Only clones with the highest/slowest off-rate were tested. All clones not belonging to a big VH-CDR3 group were tested in IHC.

Antibodies 807A-M0026-F05 and 807A-M0027-E11 did not cross-react with each other. However, both cross-reacted with antibody 807A-M0028-A07, indicating that both antibodies recognise a similar but not the same epitope.

Antibody 807A-M0028-B02 possibly recognises another epitope than antibodies 807A-M0026-F05 and antibody 807A-M0027-E11.

Example 10: Off-rate measurements

To optimise Biacore measurements, we used a Biacore chip coated with streptavidin to bind bCTD. First we analysed antibodies 3D12 and E19 for binding to the chip. We also recloned a Fab that we recovered from the pre-screening (not binding to VLDL, positive in Fab ELISA). Mab 3D12 did not bind in Biacore, probably due to low affinity. Ab E19 and non-purified Fab 1F7 (dialysed periplasmic fraction) did bind to the bCTD chip. As a control we used a channel coated with bBSA; neither antibody bound to this surface.

Off-rate ranking of selected Fabs was determined with this bCTD chip using periplasmic extracts (important for ranking clones, now and during affinity maturation studies). Table 1 shows a representative list of off-rates.

Affinity determination was determined on a low density of bCTD on the chip using purified Fab fragments.

Example 11: Immunohistochemistry

Immunohistochemistry (IHC) on the antibodies with the slowest off-rate of the identical VH-CDR3 groups and all antibodies having a different VH-CDR3 (single clones) were tested in IHC performed on frozen tissue slides.

For IHC and affinity measurement purified Fabs were used. Antibody fragments (Fabs) were expressed in bacteria (typically in 400-ml cultures, 4 hours IPTG induction) and purified from periplasmic extracts by Immobilized Metal Affinity Chromatography (IMAC). Periplasmic extracts were prepared by "osmotic shock" treatment of the bacteria. The samples were loaded on a 1ml Co-IDA column and eluted with a 0-150mM linear gradient of imidazole. Protein preparations were dialyzed against PBS and analyzed by non-reducing SDS-PAGE.

Only 3 antibodies bound as Fab on AD plaques: 807A-M0027-E11, 807A-M0028-B02 and 807A-M0026-F05.

Antibody 807A-M0027-E11 detects Alzheimer's Disease (AD) plaques in IHC. It is important that antibodies specifically recognise plaques in tissue and not to Apolipoproten E exposed in serum. Typically, an antibody of the invention binds to plaques of at least two patients having AD and to patients potentially also having systemic amyloidosis. Therefore, IHC was performed in the presence of fresh plasma. In IHC staining with 807A-M0027-E11, as phage and as Fab, in the presence of even 50% plasma, the signal was not quenched. Also the addition of VLDL in solution did not change the staining. This is in contrast with the 6C5 control antibody, directed to NTD, the signal of which was quenched by fresh plasma or VLDL solution. SFab antibody 807A-M0028-B02 stained positive on AD plaques and also astrocytes. SFab antibody 807A-M0026-F05 stained AD plaques weakly. The staining pattern for this antibody is not very strong, caused by low affinity of this antibody. The antibodies were positive in tissues of more than 1 patient.

Other sFab antibodies: 807A-M0039-C10, 807A-M0037-D01, 807A-M0046-A06 and 807A-M0039-C10 only detected astrocytes on AD brain tissue.

Example 12: Affinity measurement of sFabs binding to plaques in IHC

The three Fabs 807A-M0027-E11, 807A-M0028-B02 and 807A-M0026-F05 were extensively studied in Biacore analysis. First bCTD was coated on a streptavidin chip, then sFabs were run over the chip at different concentrations and

binding resonance units (RU) were measured. As a negative control, one channel of the chip was saturated with biotin-BSA. Figure 4A shows the analyses of sFab antibody 807A-M0027-E11 on bCTD resulting in an affinity is 47.8 nM. The affinity of sFab antibody 807A-M0028-B02 showed a similar pattern, with an affinity of 179 nM (Figure 4B). Antibody 807A-M0026-F05 has such a low affinity (in the μM range) that it is difficult to measure (Figure 4C).

In contrast, when sFabs were coupled to a CM5 chip via an anti-Fc antibody, no binding was observed for antibody 807A-M0027-E11 (see Figure 4D), nor for antibody 807A-M0028-B02.

The different results reversing the coating and analyte, in the two different Biacore measurements suggest that both, antibody 807A-M0027-E11 and 807A-M0028-B02 only bind to coated bCTD and not to bCTD in solution.

<u>Example 13: Antibody reformatting, expression and purification of IgGs</u>

Antibody (batch) reformatting to IgG1

85 clones showed specific binding to CTD as soluble Fab. Of these the 30 candidates that had been chosen for IHC studies were reformatted to complete human IgG1 antibodies.

A pool of 157 CTD-specific Fabs, that contained all 85 "soluble Fab binders", was used for simultaneous, restriction digestion based *batch-reformatting* into the human IgG1 expression vector pBh1.

The *batch-reformatting* strategy involved two cloning steps, and is illustrated in Figure 5. In the first step, complete Fab fragments are inserted into pBh1. In the second step, internal/regulatory sequences are exchanged.

To "re-identify" the initial Fabs, about 300 individual clones were analysed by DNA-sequencing. 72 of the 85 "soluble Fab binders" were found back. Notably, 29 of 30 of the prioritised candidates chosen for IHC (as phage and soluble Fab) were obtained as IgG1 constructs by *batch reformatting*.

11 of the 13 remaining "soluble Fab binders" could be reformatted individually into the human IgG1 expression vector pRh1. Identity of the reformatted antibodies to their Fab counterparts was verified by sequencing. Besides initial PCR-

amplification of the complete Fab-insert, the cloning approach is identical with the *batch-reformatting* strategy depicted in Figure 5.

Expression and purification of IgG1 antibodies

Reformatted IgG1-antibodies were expressed in transiently transfected HEK293T cells. Antibodies were purified from culture supernatant of ~5 X 10^6 transfected cells (per flask), kept in culture for about one week. Purification was carried out by Protein-A-based affinity chromatography. Purified antibodies were dialyzed against PBS and analysed on SDS-gel under reducing and non-reducing conditions.

Biotinylation of IgG antibodies

Biotinylation of antibodies was performed in PBS, incubating the purified antibodies for 2 hours with a 15-fold molar excess of Sulfosuccinimidyl-2-(biotinamido) ethyl-1,3-dithiopropionate. The level of biotin-incorporation (i.e. the average number of biotin groups per antibody molecule) was determined using the HABA [2-(4'-hydroxyazobenzene) benzoic acid] method (Pierce). Using this approach, we found that all biotinylated antibodies contained 3 to 4 biotin groups per molecule.

Example 14: Specificity tests with IgG1 antibodies

Binding of IgG1 antibodies to other species

Cross-reactivity of the human antibodies on bCTD of these species was tested to try to identify antibodies that can be studied in mouse and primate models.

The antibodies (807A-M0028-B02, 807A-M0027-E11, 807A-M0026-F05) that bound to plaques in AD tissues as sFab, were reformatted to hIgG1 and tested for their binding capacity on recombinant mouse CTD (mbCTD), recombinant primate CTD (pbCTD) and recombinant human CTD (hbCTD) that was biotinylated.

Antibodies 807A-M0028-B02 and 807A-M0027-E11 (Figures 6A and 6C) did bind to CTD of the three different species. Both antibodies bound to the same extent suggesting that the epitopes recognised by the antibodies are the same in these species.

Antibody 807A-M0026-F05 (Figure 6B) binds to pCTD and hCTD but not to mouse CTD, suggesting that the antibody is directed against an epitope that is not present in mice or that concentration used is not high enough. The low O.D. in this ELISA can be explained by the low affinity of this antibody.

Binding of IgG1 antibodies to bNTD

807A-M0027-E11 and 807A-M0026-F05 did not bind to bNTD. Antibody 807A-M0028-B02 did bind at very high concentrations (10 and 5 µg/ml). To check if this was related to specific binding, we tried to compete with 1000 times more bNTD in solution (540 ug/ml in solution, 0.5 ug/ml coated) (Figure 7). The non-specific signal did not decrease in competition. The signal is most probably due to the high amounts of antibody added, as also seen for the anti-MUC1 antibody PH1 (Figure 7).

Binding of IgG1 antibodies to VLDL

Binding of antibodies to coated VLDL was tested in ELISA. Unlike the phage and the Fabs of the plaque binders, antibodies 807A-M0028-B02 (Figure 8) and 807A-M0027-E11 are binding to VLDL to the same extent. When compared to the binding of a non-CTD binder (PH1, which is a MUC1 binder) this binding seems to be specific.

Because VLDL is composed of ApoE and a lipid, the coated VLDL could change its conformation during treatment in ELISA and it could be that the CTD is not covered by the lipid anymore. Therefore we did a competition test with the antibodies in solution, bCTD bound to the plate and an excessive amount of CTD or VLDL in solution (Figure 9). In this assay we could not see inhibition with VLDL for the human antibodies (Figure 9A and B) and little inhibition with CTD for antibody 807A-M0028-B02 (Figure 9B). In contrast, the commercial antibodies 3D12 and E19 directed against CTD were clearly inhibited by VLDL as well as by CTD. The 6C5 monoclonal directed to NTD did not bind in this assay.

These results suggest that the human antibodies (807A-M0028-B02, 807A-M0027-E11) are not binding to VLDL in solution and recognise CTD in solution to a lesser extent than coated bCTD.

Conclusion

Two of the three antibodies binding to plaques in AD (807A-M0028-B02, 807A-M0027-E11) are cross-reactive with pCTD and mCTD. The third antibody (807A-M0026-F05) does not cross-react with mCTD and binds pCTD. The antibodies of interest do not bind to NTD. Two antibodies (807A-M0028-B02, 807A-M0027-E11) bind to coated VLDL, when high antibody concentrations were used. Antibody 807A-M0026-F05 does not show these properties but this could be related to the affinity of the antibody.

<u>Example 15: Biacore analysis</u>

To compare Fab and IgG1 binding and to study the nature of binding of the human antibodies on CTD in solution, Biacore was extensively used.

Comparison of Fabs and IgGs in Biacore

Figures 10, 11, 12 and 13 summarize the results of the Biacore analysis of the CTD-specific clones 807A-M0026-F05, 807A-M0027-E11 and 807A-M0028-B02. As expected all three plaque binders bind better to bCTD on the chip as IgG1 compared with their original Fab format. IgG1 Antibody 807A-M0027-E11 binds 3 times better than antibody 807A-M0028-B02 while antibody 807A-M0026-F05 binds with a very low micro molar avidity to the chip.

The avidity measured (Table 2) is higher when measured 50 seconds after the injection of the antibody is stopped as compared with the avidity measured immediately after injection stop. This difference is probably due to the rebinding of the antibody to the chip when free bCTD is available on the chip.

Binding of antibodies to captured CTD

Because of the inconsistency of VLDL ELISA in which the human IgG1 antibodies bind to coated bCTD/VLDL but not CTD/VLDL in solution, additional Biacore experiments were performed (see also Biacore on Fabs). In these experiments bCTD or ApoE was captured. First anti-hFc antibody was coupled to the chip, followed by binding of the specific antibodies, followed by the injection of bCTD or ApoE.

Figure 12 shows that Fab antibody 807A-M0027-E11, indirectly coupled to the chip, does not bind bCTD (280 nM). The same curves (data not shown) were obtained by using Fab antibody 807A-M0028-B02 and when IgG1 of both antibodies was used. The test was performed with ApoE originating from human serum. Only a very small amount of ApoE (17 RU, < 1%) was bound to the antibody (Fig 13).

Conclusion

In Biacore, IgG1 antibodies 807A-M0027-E11 and 807A-M0028-B02 bind to coated bCTD with nM avidity while IgG1 antibody 807A-M0026-F05 binds with µM avidity. Affinity measurements on antigen in solution do show that both antibodies 807A-M0027-E11 and 807A-M0028-B02 do not capture bCTD nor ApoE efficiently. These results confirm the results seen by VLDL/CTD competition ELISA: antibodies 807A-M0027-E11 and 807A-M0028-B02 bind better to coated bCTD than to CTD in solution.

Example 16: Additional testing to study binding of antibodies to natural CTD, to peptides

SDS-PAGE analysis of the purified hApoE

The purified, hApoE was analyzed by reducing SDS-PAGE followed by Coomassie staining. As expected, the protein migrated as one major band of ~ 35 kDa, but there was also a broad band at ~ 70 kDa and a faint smear from 70 kDa to ~ 200 kDa. Both the 35 kDa band and the higher molecular weight species were shown by western-blot to contain hApoE.

Immunoprecipitation of purified hApoE

Purified hApoE was immunoprecipitated with 807A-M0028-B02, 807A-M0027-E11 and 807A-M0026-F05. As a positive control, we used E19, a goat anti-hApoE antibody. As a negative control, we used the PH1 antibody. Untreated, purified hApoE was also included as a reference. The samples were analyzed by SDS-gel, the proteins were transferred to a nitrocellulose membrane and hApoE was detected by western-blot.

As expected, the E19, the 807A-M0028-B02 and the 807A-M0027-E11 antibodies were able to specifically immunoprecipitate hApoE, although not very efficiently. Interestingly, the E19 antibody seemed to be specific for the 35 kDa band, whereas the 807A-M0028-B02 and 807A-M0027-E11 antibodies were more specific for the high molecular weight species. The 807A-M0026-F05 was by contrast unable to immunoprecipitate hApoE, probably due to the low affinity of the antibody.

Immunoprecipitation of cell lysates

Cell lysates of PBMC were immunoprecipitated using 807A-M0028-B02, 807A-M0027-E11 and 807A-M0026-F05, as well as E19 (positive control) and M43G5, M43F8, PH1, A2, herceptin and a human IgG1 Kappa Myeloma antibody (negative controls). Samples were analyzed by SDS-PAGE under reducing conditions followed either by silver staining or Western-blot.

Only E19 immunoprecipitated a band of the expected size (i.e. ~ 35 kDa). The material immunoprecipitated with E19 also gave a very faint signal in Western-blot. This suggests that some hApoE is captured by E19 in the cell lysates, although this finding must be regarded with care since (1) 807A-M0028-B02 and 807A-M0027-E11 did not immunoprecipitate any hApoE from the cell lysates, (2) 807A-M0026-F05, which was shown to be unable immunoprecipitate hApoE, also gave a weak signal in Western-blot and (3) A2 and 807A-M0043-F08, two irrelevant antibodies, gave strong signals in Western-blot.

Importantly, the three antibodies investigated here (807A-M0028-B02, 807A-M0027-E11 and 807A-M0026-F05) did not seem to immunoprecipitate any major component of the cell lysates. The background for 807A-M0027-E11 was a little bit higher, but not higher than e.g. PH1. These results indicate that the overall specificity of the antibodies, 807A-M0028-B02, 807A-M0026-F05 and 807A-M0027-E11, is due to the binding to CTD.

Immunoprecipitations of VLDL

Binding of antibodies to VLDL was also tested by immunoprecipitation. 10% VLDL was used in these tests. As detection antibody 6C5 was used. After 5 minutes development of a Western blot with the ECL method, no ApoE was detected

for the human antibodies of interest. After overnight development antibody 807A-MO027-E11 and 807A-M0028-B02 were detected as faint bands when compared with the VLDL control (non-immunoprecipitated VLDL, 10% of the amount that was used for immunoprecipitation). Immunoprecipitation with the 6C5 antibody showed a more extensive band than with the human antibodies.

Discussion

807A-M0028-B02 and 807A-M0027-E11, but not 807A-M0026-F05, preferentially immunoprecipitate a high molecular weight from of hApoE purified from plasma. The nature of these species remains unclear, although it is clear that they contain hApoE and must form very stable complexes. These antibodies do not interact significantly with major cellular components. Immunoprecipitation of VLDL with the antibodies is possible, although the amount might be very low.

<u>Example 17: *In vivo* studies</u>

Mice were bred to express in the brain the human gene for amyloid precursor protein (APP): Swedish mutation K670N,M671L, APP Line 2576, driven by the hamster prion promotor (Hsia *et al* Science 1996, 274:99-102), alone or in combination with a mutated human presenilin 1 (PS1):M146L driven by the platelet derived growth factor (Duff *et al*, Nature 1996, 383 (6602):710-3, Holcomb *et al*, Nature Med 1998, 4:97-100).

For studies requiring expression of human ApoE mice were bred that either expressed the human ApoE4 driven by the glial fibrillary acidic protein (GFAP) promotor with or without the mouse ApoE gene knocked out (Sun *et al*, J Neurosci, 1998, 18:3261-3272) in combination with humAPP:Swe and humPS1:M146L or humAPP:Swe only.

Monoclonal antibodies (mAb) of the human immunoglobulin G1 (hIgG1) isotype to C-terminal domain (CTD) of Apolipoprotein E (ApoE) were injected intra-peritoneally at a concentration of e.g. 10 mg/kg in non transgenic or transgenic mice. Some mice were injected once and sacrificed 2 days after injection and some were injected twice, with the repeated dose injected after 2 days, and then sacrificed after an additional 2 days i.e. 4 days after the initial injection. The concentration of injected antibody was monitored by ELISA to CTD binding hIgG. Brains from mice

injected with a streptavidin specific monoclonal antibody served as negative controls. The sampled brains were immediately frozen to –70°C and then subjected to freeze sectioning.

Staining for presence of human IgG in brain sections showed homogeneously stained plaques evenly spread trough cortex and hipocampus. No other brain structures showed staining after *in vivo* exposure. In contrast, several structures along with the amyloid plaques were stained after *ex vivo* exposure. 70% of the plaques that were accessible for staining *ex vivo* by anti-ApoE CTD antibody or with a mAb to Ab were stained after the 2-day (n=3) or 4-day (n=3) *in vivo* exposure with the anti-ApoE CTD antibody. The *in vivo* exposure for 2 or 4 days at the given dose did not saturate the available binding sites as indicated by the additional staining intensity obtained after *ex vivo* addition of more anti-CTD antibody.

Antibodies, as macromolecules in general, do not pass freely over the blood brain barrier (BBB). The passage of IgG is considered to be very limited and concentrations in CSF under 0.5% of the plasma concentration has been reported (Elovaara *et al*, 1987 Eur Neurol 26:229-34, Ganrot & Laurell 1974, Clinical Chemistry 20:571-3). Staining for presence of the intraperitoneally injected human IgG in brain sections revealed that the ApoE CTD specific antibody reached the cerebral plaques evenly throughout the different brain regions in these transgenic mice indicating sufficient BBB passage for staining by immunohistochemistry (IHC) technique. Alzheimer's disease (AD) plaques are complex structures varying in size and density. The cerebral amyloid plaques found in these transgenic mice are considered to represent the small diffuse and medium size plaques found in AD. The plaques were homogeneously stained indicating that the mAb did not only reach the outer layer of the plaques but penetrated the whole plaque structure. 70% of the plaques accessible for staining *ex vivo* were stained after the *in vivo* exposure. Considering that the dose of 10 mg/kg administered interperitoneally did not saturate the available plaque-binding sites, a non-saturated level of antibodies may still result in an antibody mediated plaque breakdown by FcR bearing phagocytic cells.

Example 18: Reformatting of Antibodies to mouse IgG2a

For *in vivo* testing the variable regions of antibodies 807A-M0028-B02, 807A-M0027-E11, 807A-M0026-F05 and a control antibody (anti-Streptavidin clone

A2) were recloned into vector that contains mouse IgG2a constant regions of the heavy chain and mouse Ckappa and the variable regions of antibodies.

These clones were transferred from a human IgG1 expression vector (pBh1) to a construct for expression of mouse IgG2a antibodies (pRmk2a), that besides the constant heavy chain region, also contains the mouse constant kappa light chain gene. The VL and VH regions were lifted from the human IgG1 expression plasmid via PCR, and cloned sequentially into pRmk2a. VL was inserted as an ApaL1/BsiW1 fragment, 3' of the antibody leader and 5' of the constant kappa gene. In case of the VH region, the 5' adjacent IRES motif was also included in the PCR amplification product; an Asc1/Nhe1 fragment was inserted in pRmk2a. Integrity of the constructs was verified by DNA sequencing. The cloning strategy is depicted in Figure 14.

Example 19: Preparation of Peptides

10 peptides (length 16 amino acids) covering the full ApoE CTD were synthesized.

The peptides contain an 8 amino acid overlap between each other as shown in Figure 15. The peptides contain an S-S Biotin group that enables binding to Strepatavidin (magnetic beads selections). In addition each peptide contains a Cystein that can be coupled to a carrier protein (BSA).

Peptides were solubilised in dimethylformamide (DMF), and subsequently diluted in water. All peptides, except peptide 4 were soluble in DMF at a concentration of less then 10%. Coupling was performed in 10% DMF for all peptides, except for peptide 4 which was coupled in 30% DMF. An excess of maleimide-activated BSA was used to bind to the peptides. After incubation an excess of Cystein was used to occupy possible free cysteins. The BSA coupled peptides (bpeptide-BSA) were used for selections.

Example 20: Binding of antibodies to overlapping peptides

Antibodies 807A-M0026-F05, 807A-M0028-B02 and 807A-M0027-E11 identified in Example 5 were tested for their binding to overlapping peptides. This was done firstly to test which peptides could be preferentially used in further selections: bpeptide-BSA or bpeptide and secondly to see whether the antibodies selected in Example 5 were binding to the overlapping peptides and if so, whether

the epitopes they recognised were different and supported the epitope mapping by competition results as performed in Example 9. Both human antibodies and mouse antibodies were compared in this way.

For the human antibodies, peptide mapping was performed with IgG1 as well as with the phage displayed Fab. For example, clone 807A-M0026-F05 recognised bpeptide-BSA 4 and 8 as phage Fab fragment as well as whole IgG1. The ELISA was not sensitive enough to show binding to bpeptide 4 and 8 for this antibody. Therefore, we decided that it would be best to start the selections on bpeptide-BSA to capture the majority of peptide binders and then, if necessary, use the bpeptide in a later round of selection. Remarkably, antibody 807A-M0026-F05 bound better to CTD than to peptide compared with the murine monoclonal antibodies. Antibody 807A-M0028-B02 bound to peptide 4. Antibody 807A-M0027-E11 did not significantly bind to any of the overlapping peptides.

In Example 9 we found that the epitope recognized by antibody 807A-M0026-F05 and antibody 807A-M0027-E11 was covered by a large group of antibodies. Both antibodies did not compete with each other. Since the affinity for CTD for antibody 807A-M0026-F05 is very low as compared with antibody 807A-M0027-E11, one would expect that, if they recognise the same epitope, antibody 807A-M0027-E11 would have bound more strongly to peptides 4 and 8 than antibody 807A-M0026-F05. Therefore, one could conclude that both antibodies recognise related but not identical epitopes. Antibody 807A-M0028-B02 bound to peptide 4 and was different from the antibody group of the two other antibodies in competition epitope mapping and could recognise a different epitope.

We also tested control mouse antibodies on overlapping peptides. Antibodies 3H1, 12D10 and E19 bind to peptides 3, 10 and 5+10 respectively. In contrast with the human antibodies, all control antibodies bind to about the same extent to peptide and bCTD.

Example 21 : Selections and screening on peptides

Three rounds of selection were carried out on 10 overlapping biotinylated peptides conjugated to BSA (b-peptide-BSA) and one round of selection on the corresponding biotinylated peptides (b-peptide). Selection was performed on 10 individual peptides using Streptavidin-magnetic beads. To handle this high number

of selections in between selection rounds no titration of input/output was performed (liquid amplification).

The procedure used is set out in Figure 16. First, three rounds of selection for binding to bpeptide-BSA was carried out using the automated Kingfisher system. Pre-screening of round 2 and 3 with Fab-displayed on phage showed that the frequency of positive clones was low in round 2 and that many clones were binding to BSA in round 3, despite extensive depletion and subtraction on BSA. Most likely the binders were directed to the linker molecules on the BSA that we used for coupling the peptides. Therefore another round 3 selection on bpeptides was performed. For this selection background binding was negligible.

To reduce sequencing efforts, phage-Fab clones were batch reformatted to produce sFabs and the large screening of the clones (ELISA and sequencing) was performed at sFab level. 307 antibodies were found positive in ELISA of which 46 were unique as was determined by sequencing as shown in Tables 11 and 12.

Example 22: Selections and screening on fibrils and peptides

Two rounds of selection were carried out on fibrils originating from an organ with amyloid plaques, 2 rounds on 10 corresponding b-BSA-peptides and 1 round on 10 corresponding b-peptides.

The procedure used is summarised in Figure 17. Round 3 and round 4 were performed on individual bpeptides-BSA. After pre-screening few positive clones were found. Therefore a fifth round of selection was carried out using bpeptides. In a pre-screen, positive clones for peptide 4 and 8 were found. For these two selections we screened sFab after batch reformatting. In total 390 sFabs were screened, 109 were positive in ELISA and 4 clones were unique. The amino acid sequences of the VH and VL chains of these unique clones are shown in Tables 11 and 12. In these strategies most clones did show unique VH-CDR3s (Table 12), and high enrichment was found: one clone was enriched 148 times and was found in strategy B1 as well as in strategy B2. This is in contrast with selections of Example 5.

Few clones (Table 13) bound to bCTD, which makes them unique as compared to results in Example 20 where bCTD binders bound weakly or not at all

to peptides. In the selection campaigns of Examples 21 and 22 no Fabs were binding to VLDL nor to NTD.

11 Fabs were found positive in IHC, 10 of these Fabs originate from the selection campaign of Example 11 and one originates from the selection campaigns of both Examples 21 and 22.

Example 23: Selections and screening on ur-bCTD and peptides

Two rounds of selection were carried out on urea treated biotinylated CTD (ur-bTD) followed by two rounds on fibrils originating from an organ with amyloid plaques. The procedure used is summarised in Figure 18.

Two rounds of selection on urea-CTD antigen were performed followed by 2 selection rounds on fibrils 1 from an AD patient. In this strategy pre-screening was done after the 3rd and 4th round of selection. Frequencies were 82/95 and 83/95 respectively. During pre-screening we used ur-bCTD and bCTD and no difference was found between both types of antigen. After the third selection round, many clones were retained that bound to bCTD but not to fibrils while after 2 rounds of selection on the fibrils (4rd round of selection) the chance that these binders are retained was less. Therefore, large scale screening was performed on the 4th round of selection. 950 sFabs were screened on ur-bCTD. 233 clones were positive in ELISA as soluble Fab, 83 were unique. The amino acid sequences of the VH and VL chains of these uniques Fabs are shown in Tables 14 and 15. In VLDL ELISA, 5 Fabs bound were positive or had questionable binding.

No Fabs bound to bNTD.

Many Fabs belonged to large families of identical VH-CDR3 groups; one individual antibody was enriched 247 times. Testing in IHC was performed on individual clones (not belonging to a large VH-CDR3 group) and clones belonging to a large VH-CDR3 group that were selected for a slow off-rate.

In a first IHC screen, 6 Fabs bound to plaques of AD patients. The properties of the bCTD binders are summarised in Table 17.

Example 24: Production of candidate clones as soluble Fabs for IHC

Fabs were produced for further analysis. ELISAs were performed on periplasmic fractions of 100 μl cultures. Biacore off-rate measurement on

periplasmic fractions of 50 ml cultures of all clones found in tables with Biacore results.

About 90 soluble Fab proteins were produced for testing in IHC. At least 10µg was required for initial analysis. Because of wide variation in soluble Fab expression levels, the protein had to be purified either from the peripheral extract of 50ml bacterial cultures, or from peripheral extracts of 400ml cultures, by IMAC chromatography, using 96-well filter plates and a vacuum manifold. The yields mainly ranged between 10-100µg.

Large scale production of IHC positive soluble Fabs

15 of the candidate Fabs turned out to be positive / potentially positive in IHC on plaque tissue. Of these clones more soluble Fab protein were prepared for additional testing.

Soluble Fab proteins were prepared from the periplasmic extracts of 400ml bacterial cultures.

Example 25: Epitope mapping, comparison with preliminary IHC results

High throughput Fab screening was performed on the peptides they were selected on and also on a non-overlapping peptide as a control. In epitope mapping all peptide positive antibodies were screened for their binding reactivity to all other peptides. Table 13 contains detailed results.

Two Fabs bound to all peptides (most likely to the BSA-linker) and were considered as non-specific.

No Fabs were identified that were specific for peptide 5 and 10 (although the pre-screening Fab on phage showed some positive binders).

49 different Fabs bound specifically to peptides. 39 of the specific Fabs only bound to the peptide they were selected on. One Fab selected on peptide 1 also recognised peptide 6. 3 Fabs originating from the peptide 4 selection also bound to peptide 9 and 3 Fabs originating from the peptide 9 selections recognised peptide 4. Further, 3 peptides selected on peptide 8 also bound to peptide 4. Only 9 Fabs bound also to bCTD. This suggests that most of these Fabs bind to an epitope that is not present in the recombinant bCTD and recognise another (possibly stretched) structure that could potentially also be found in plaques. The region that covers

peptide 3 and peptide 4 is recognised as a 'selection dominant epitope', containing 39 of all 49 specific Fabs and 8 of 9 bCTD binders. Interestingly, this area of ApoE is thought to be involved in the binding to VLDL particles.

Hypothesis taking into account for the IHC screen

39 of the 49 specific Fabs bound to peptide but not to bCTD. This suggests that these Fabs are not likely to recognize natural Apo-E contained in for example VLDL particles. If such a Fab would bind in IHC it could indicate that such an epitope is unique and only found on plaques and that these Fabs would be important leads for further investigation.

Indeed, preliminary IHC data shows that four of these Fabs possibly bind to tissue in AD patients. These four Fabs all bind to peptide 4 and not to the overlapping peptides, suggesting that they recognise similar (overlapping) epitopes (group 1), probably epitope containing amino acids of LVEDMQRQ or a secondary structure only present in peptide 4 and plaques.

Two other Fabs, positive in IHC and selected on peptide 4, bind to peptides 4 and 9 and to a conformation that is not present or not as prevalent in bCTD. Possibly the epitope for these two antibodies include sequence MQRQWAGL (group 2).

Another Fab possibly positive in IHC, selected on peptide 9, only recognises peptide 9 and not overlapping peptide nor bCTD and could recognise either the epitope WAGLVEKV or a conformation only present in peptide 9 (group 3) and plaques.

10 of 49 specific Fabs bind to peptide and bCTD, suggesting that these antibodies recognize a more conformational epitope.

One Fab binds to peptide 1, 6 and bCTD. This epitope (RTRDRLDE) is not predicted to be inside of the binding site of VLDL (group 4).

Two antibodies, obtained from selections on peptides 4 and 9, recognise both peptides (epitope MQRQWAGL) and CTD and therefore are different from Fabs of group 2 (group 5).

One antibody, selected to peptide 8, binds to peptide 4, 8 (epitope WFEPLVED) and bCTD (group 6).

Thus, according to this hypothesis the Fabs identified in Examples 21 and 22, can be divided into six different groups of Fabs that each recognize distinct epitopes.

Example 26: Biacore off-rate analysis of the Fabs identified in the strategies of Examples 21 and 22

Off-rate analysis of soluble Fabs was performed on all 10 peptides. Periplasmic fractions from all unique Fab of Examples 21 and 22 were made and tested. The results confirm the epitope mapping by ELISA.

Fabs that bind to more peptides and/or to bCTD most often show the about the same off-rate for those molecules. In contrast, RU's (measure for the amount of antibody bound) are often highest for the peptide to which the Fabs were selected on.

The strategies of Examples 21 and 22 did not result in the identification of antibodies belonging to large families of identical VH-CDR3s. Therefore, off-rate measurements were not used as a criterion for IHC.

Example 27: Epitope mapping of the antibodies of Example 23 and comparison with preliminary IHC results

In the automated screening, Fabs were screened for their binding reaction towards ur-bCTD and streptavidin BSA as negative control. No antibodies bound to NTD. Table 16 contains detailed results.

In total 81 different antibodies, binding to ur-bCTD and CTD were found. 5 of those antibodies bound to coated VLDL. None bound to bNTD. 20 antibodies, also bound to peptide. As in the strategies of Examples 21 and 22 we found a selection dominant epitope around peptides 3 and 4.

Five antibodies of the 81 bound in IHC. Only one of these antibodies bound to peptide. This antibody binds with low RU (Biacore) to peptide 4 and high RU. Interestingly, this antibody was also found using the strategies of Examples 21 and 22. The other four antibodies could be compared with antibodies 807A-M0028-B02 and 807A-M0027-E11 of Example 5.

Example 28: Reformatting of candidate Fabs to human IgG1

Most of the IHC positive clones described above can be individually reformatted to the Dyax hIgG1 expression construct pBh1 in two restriction endonuclease based ("cut and paste") cloning steps (see Figure 5).

With the exception of the amber-stop containing clones, 807B-M0079-D10 (807B-M0027-D08) and 807B-M0081-A11 (807B-M0081-F12), and the clone

807B-M0009-C03, reformatting of Fab to IgG was carried out and the IgGs transiently expressed in Hek293T cells.

The amber-stop mutation in the CDR2 of 807B-M0079-D10 is corrected on the "phagemid level", before the clone is reformatted using the procedure outlined above.

The amber-mutation at the 5'-end of VL of 807B-M0081-A11 is repaired using a different reformatting strategy / "PCR-based reformatting to the hIgG1 expression construct pRh1". Due to the fact that the amber-stop mutation lies within the sequence of our "CJ-kappa-lifting primer", the stop mutation is corrected during PCR amplification of the Fab fragment. The cloning strategy of PCR/Fab fragments to pRh1 is the same as the "cut and paste" approach to pBh1.

Example 29: Conclusions

229 candidate Fabs binding to CTD were isolated from a variety of selection procedures with Dyax' human Fab300 library. Two of the procedures included selections on peptide (Examples 21 and 22). In the selection procedure of Example 23 the successful selection of Example 5 was reversed by first selecting on ur-bCTD and then on fibrils. Also in contrast with Example 5, we did not screen phage but first performed a batch recloning from phage Fab to Fab.

Very few (five) were reactive, as Fab antibody, with coated VLDL. No Fab was positive for bNTD.

In the strategies of Example 21 and 22, some clones were enriched and we observed VH-CDR3 groups with few individual clones.

Fab antibodies from the strategies of Example 21 and 22 recognise a selection dominant epitope around peptides 3 and 4. In the strategy of Example 23, the same dominant epitope is found.

In IHC screening, 15 antibodies were found positive for binding to plaques in IHC in a first screen. An overview of the characteristics of the Fab clones positive in the first IHC screen is shown in Table 17.

Example 30: Properties of IgG1 of the 15 Fabs positive in IHC

The 15 Fabs which were positive in Fab-IHC were reformatted to IgG1. Of these antibodies, nine were found positive in IgG1-IHC.

Almost all IgG antibodies bound to peptide in peptide epitope mapping. This is probably due to the higher avidity of the IgG1 as compared to the Fab. For the antibodies slected on peptide 4 which only bound to peptide 4 as Fab, some also bound to peptide 9 as IgG. For the antibodies originally from the screen described in Example 23, the IgG antibodies bound to peptides 4 and 8, peptide 9 or peptide 7 only one antibody, 807B-M0083E11, did not bind to any peptide.

Human CTD, mouse CTD and primate CTD were compared to each other. Several IgG antibodies bound to bCTD of each of the three species.

Although there was no binding to coated VLDL in Fab ELISA, in IgG1 ELISA several but not all antibodies bound to VLDL.

All IgG antibodies showed an improved binding compared to Fabs in Biacore analysis when bCTD was coated on the chip.

The IgG1 results are summarised in Table 18.

Example 31: Effect of VLDL on binding of 807A-M0028-B02 to CTD in amyloid deposits

To analyse binding of 807A-M0028-B02 to CTD in amyloid deposits in presence of lipoprotein particles, immunohistochemistry was performed in presence or absence of VLDL to see whether the presence of VLDL would lead to a decreased staining intensity of plaques.

The 807A-M0028-B02 antibody was serially diluted and mixed with VLDL prior incubation of both APP/PS1 and human AD brain sections. No quenching of signal was observed even at the lowest antibody concentration (0.04 µg/ml). In contrast, a commercial antibody to CTD (3H1) was completely quenched already at a high concentration of antibody (5 µg/ml) (Figure 20).

Example 32: Effect of 807A-M0028-B02 antibodies on phagocytic activity of microglial/ macrophage cells

To evaluate the effects of 807A-M0O28-B02 on phagocytosis activity, a phagocytosis assay of CTD immobilized on Avidin-coated FluoSphere fluorescent microspheres (Molecular Probes) was developed.

The biotinylated CTD immobilized on 1.2 µm yellow-green latex FluoSpheres® NeutrAvidin™ labeled microspheres (Molecular Probes Europe BV,

Leiden, The Netherlands) were resuspended with different concentrations of IgG converted clone 807A-M0028-B02 diluted in OPTI-MEM medium supplemented with 1% BSA (Tissue culture tested, Invitrogen AB, Sweden) and 2% ITS-X (Gibco, Invitrogen AB, Sweden) serum supplement (CM) and incubated for 30 min at +4°C.THP-1 cells (10^6 cells ml^{-1}) in CM with or without 2.0% NaN_3, the inhibitor of phagocytosis, were added at a ratio of 1:100 (cells:beads). To allow binding to F_c-receptors, cells were synchronised at +4°C for 20 min and the cell-free beads were removed by low-speed centrifugation (200g, 10 min, +4°C). The cell pellets were resuspended in CM and incubated at 37°C for 40 min in a CO_2-incubator. After trypsinisation, the cell suspension was taken in sterile conditions, layered over a 7.5% bovine serum albumin (BSA) cushion and centrifuged at 150×*g* for 10 min at +4°C, to remove non-internalised beads. The cell pellets were resuspended in 0.3 ml of 2% PFA in PBS. The results were expressed as the percent of the control, i.e. amount of phagocytic cells containing two or more beads in presence vs. in absence of antibodies, as determined by flow cytometry. A FACScan™ (Becton Dickinson, San Jose, CA) with an air-cooled argon laser providing an excitation at 488 nm was used. A total of 10000 events were acquired for each sample and stored in the list mode data format. The fluorescence emission was collected at 520 nm (FL1) for the phagocytosis. Data collection and analysis were performed with a Consort 30 system and LYSIS-II program. The data were analyzed, once displayed as two-parameter complexity and cell size, in the process of gating and as fluorescence (FL1) frequency distribution histogram to analyze the phagocytosis. EC50, the concentration that induced 50% increase of phagocytic activity was determined for each antibody tested using dose–response curves built with the percentages of phagocytic activity, versus the range of concentrations (0,01-5 μg/ml). Then, the EC50 were extrapolated from these curves and used to compare the relative efficiency of phagocytic stimulation of the antibodies. The IgG converted clone 807A-M0028-B02 demonstrated high efficiency (EC50=34±15 ng/ml) to stimulate THP-1 cells. The results thus indicate that 807A-M0028-B02 specifically directed the *in vitro* phagocytic uptake of CTD-bearing beads by human macrophage/microglia-like cells in a concentration-dependent fashion.

Example 33: Germ line correction of clones found in selections described in Example 4, Example 21 and Example 22

Of the antibodies described in Table 18, five of them (807A-M0028-B02, 807B-M0004-H03, 807B-M0009-F06, 807B-M0004-A03 and 807B-M0079-D10) have been investigated further. Somatic mutations in the variable part of the light chains of these antibodies have been found in all clones. Some of the clones also contained mutations in the constant part of the light chain (Table 21). Sequence alignments with genomic and known germline sequences have been performed, and the correct amino acids have been identified (indicated in bold in Table 19 and Table 20). The VL chains of the corrected clones are described in Table 19, and the constant parts of the IgG are described in Table 20.

To ensure that the IgG molecules are germline, the somatic mutations are corrected at the DNA level in the five antibodies, and all five germline-corrected IgG1s were expressed (transiently in HEK 293T cells). Comparative binding analysis was performed in Biacore (Example 34), CTD-ELISA and IHC to ensure that the germline-corrected antibodies are still functional. The results are summarised in Table 23.

Example 34: Biacore analysis of germ line corrected IgGs

The germline-corrected clones described in Tables 19 and 20 were analysed in Biacore. The analysis was performed by running the IgGs at different concentrations over a surface with coated bCTD. A surface with a biotinylated control IgG was used as a negative surface.

Biacore analysis of clones 807A-M0028-B02, 807A-M0028-B02.1 and 807A-M0028-B02.2 showed that the three IgG molecules bind to bCTD with similar kinetics (similar on-rate and off-rate). The affinity of the IgG is not significantly altered. The same results were obtained when comparing 807B-M0004-A03 with 807B-M0004-A03.1.

Biacore analysis of 807B-M0004-H03 and 807B-M0004-H03.1 indicated that 807B-M0004-H03.1 binds with a different kinetic to the parental clone. However, this did not influence the affinity value significantly. The clone 807B-M0009-F06.1 had lost its binding capacity to bCTD as shown in CTD-ELISA and Biacore analysis and CTD-ELISA.

Example 35: Binding of antibodies to ApoE-CTD by ELISA

Antibodies were screened for ApoE-CTD binding capacity using a coated ApoE-CTD ELISA. Human, marmoset or murine ApoE-CTD was coated on a microtiter plate followed by incubation with test antibodies. After this, the amount of antibody bound was determined by detection with secondary HRP-antibody and tetramethylbenzidine (TMB) substrate. ApoE-CTD binding gives a high signal measured as optical density (OD) in the ELISA. Binding of 807A-M0028-B02, 807A-M0028-B02.1 and 807A-M0028-B02.2 are exemplified in Figure 22. Results are disclosed in Table 22.

Example 36: Binding of antibodies to human lipoproteins

Antibodies were screened for lipoprotein binding capacity using a coated VLDL ELISA. Human VLDL was coated on a microtiter plate followed by incubation with test antibodies. After this, the amount of antibody bound was determined by detection with secondary HRP-antibody and tetramethylbenzidine (TMB) substrate. VLDL binding gives a high signal measured as optical density (OD) in the ELISA. Binding of 807A-M0028-B02, 807A-M0028-B02.1, 807A-M0028-B02.2, 807B-M0004-H03.0, 807B-M0004-H03.1, 807B-M0004-A03 and 807B-M0004-A03.1 are exemplified in Figure 23. Results are disclosed in Table 22.

Example 37: *In vivo* binding of 807A-M0028-B02, 807B-M0004H03, 807B-M0004-A03, 807B-M0079-D10, 807B-M0009-F06 to mouse cerebral plaques and *in vitro* binding of Fab clones to human AD cerebral plaques

In vivo binding of 807A-M0028-B02, B807B-M0004H03, 807B-M0004-A03, 807B-M0079-D10 and 807B-M0009-F06 to cerebral plaques (immunodecoration) was demonstrated by i.p. or i.v. injections into APP/PS1 transgenic mice (Figures 24 and 25). Immunodecoration was observed already two days after a single dose administration of 10 mg/kg of 807A-M0028-B02. Binding of 807A-M0028-B02, B807B-M0004H03, 807B-M0004-A03, 807B-M0079-D10 and 807B-M0009-F06 was only observed on plaques while no staining of astrocytes or any other brain structure was detected (Figures 24 and 25). It was demonstrated that a substantial number of plaques were immunodecorated with respective clones by

comparing the total plaque burden in each mouse that was determined by staining with a monoclonal antibody to Aβ (6E10) on adjacent sections.

In vitro binding of affinity maturated clones to amyloid plaques in human AD brain sections was also demonstrated by Immunohistochemistry (Figure 25)

In vitro binding of different wild type clones in human AD plaques is visualised in Figure 26.

<u>Example 38: Affinity maturation of 807A-M0028-B02, 807B-M0004H03, 807B-M0004-A03, 807B-M0079-D10, 807B-M0009-F06 by VH-CDR3 spiking</u>

Spiking mutagenesis was used to introduce low level mutations over the full length of the VH-CDR3 of each of 807A-M0028-B02, 807B-M0004H03, 807B-M0004-A03, 807B-M0079-D10 and 807B-M0009-F06 in the context of the original wild-type residues (see Figure 29). PCR was carried out using an oligionucleotide carrying a region of spiked diversity over the length of the VH-CDR3 sequence of each antibody bracketed by regions of homology with the target V gene in the FR3 and FR4 regions together with a specific primer homologous to the FR1 region capable of annealing to the 5' end of the target V gene.

For antibodies 807A-M0028-B02, 807B-M0009-F06 and 807B-M0004-H03, diversification of the VH-CDR3 was realized through a one step PCR amplification. This PCR was performed using a 5' primer complementary to the light chain constant region and the 3'specific spiked oligonucleotides. The PCRs were then performed in a volume of 50μl using the advantage 2 PCR enzyme system (Clontech) and 10pmoles of each primer for 25 cycles (1 min at 95°C, 1 min at 60°C, and 2 min at 68°C). A primer concentration of 100nM was chosen in order to cover the entire diversity carried by the spiked oligonucleotides. 100 to 200 reactions were needed to obtain ~6 μg of PCR products. All products were purified using the GFX purification kit (Amersham).

The resulting PCR products of 730bp contain an internal XbaI site and an BstEII site, incorporated in the oligonucleotides. These sites were used to clone the products into a display vector.

Diversification of the VH CDR3 of 807B-M0004-A03 and 807B-M0079-D10 was realized in two steps: after primary amplification as described above, the

resulting PCR products of 730bp were re-amplified with a combination of a 5'end nested forward primer, appended with a SfiI site, and a 3'end Nhe1-tagged CH1 reverse primer. The PCRs were then performed in a volume of 50µl using the advantage 2 PCR enzyme system (Clontech) and 10pmoles of each primer for 20 cycles (1 min at 95°C, 3 min at 68°C); 100 to 200 reactions were needed to obtain ~6 µg of PCR products. The number of cycles was kept quite low (20 cycles) in order to maintain maximal diversity, introduced in the first PCR step. To ensure again maximal diversity, for each reaction, 50ng of the first PCR product was used as template to initiate the second PCR reaction. All products were purified using the GFX purification kit (Amersham).

The PCR products and vector backbones were digested using 50U/µg DNA of either *XbaI* and *BstEII* (for 807A-M0028-B02, 807B-M0009-F06 and 807B-M0004-H03) or *Sfi*I and *Nhe*I (for 807B-M0004-A03 and 807B-M0079-D10).

The resulting cleaved products (both vectors and PCR fragments) were gelpurified, 1.6µg of each DNA fragment was ligated into 10µg of similarly cut phagemid vector backbone using T_4 DNA ligase (NEB) and the ligation mixture for each spiked library was introduced into *E. coli* TG1 cells by electroporation.

Phagemid particles were rescued from the libraries using helper phage M13-KO7 (Marks *et al.*, (J. Mol. Biol. 222, 581 (1991)) using enough bacteria from each library for inoculation in order to represent each clone at least once.

The diversity in the VH-CDR3 of each library was evaluated by sequencing. 96 isolates were randomly picked for each library and the VH-CDR3 regions were sequenced and compared to the reference wild type VH-CDR3 sequences.

For clones of 807B-M0079-D10 and 807B-M0004-A03 the full VH sequence was determined and compared to the VH reference regions outside VH-CDR3.

A selection procedure consisting of two rounds of selection was used to preferentially enrich the higher affinity clones in the library over the lower affinity clones. The first round of affinity selection was carried out using a reduced antigen concentration relative to the concentration used to select the wild-type antibody. The optimum reduced antibody concentration was determined empirically using the wild-type antibody and a control antibody. The second round was performed at a further reduced antigen concentration in the presence of competing soluble Fab or IgG. The selection conditions used are detailed in Table 3.

To determine if enrichment of antigen positive clones had occurred, 46 randomly picked clones from before and after the first round of selection for each antibody to be matured were tested in an antigen ELISA. In all cases, enrichment of antigen positive clones after just one round of selection was observed.

After selection, the geneIII stump was removed from the vector to allow soluble Fab expression. 200 clones were randomly picked, screened by ELISA and their heavy chains sequenced. The Dyax WEBPHAGE database was used to link ELISA data to the respective sequences. The VH-CDR3 sequences of the clones found to be positive in the screening assay (OD signal = 3x background) were analysed further.

For the clones 807B-M0079-D10 and 807B-M0004-A03 the full VH region was amplified and cloned and so the full VH sequence was obtained and compared to the VH reference sequence in order to check for any mutations outside VH CDR3. Clones containing mutations in framework regions were discarded but clones with mutations in the VH-CDR1-CDR2 were kept.

The results of amino acid frequency analysis of selected clones are presented in Tables 24 to 30. In the VH-CDR3, some amino acid positions are very conserved whilst others are frequently mutated.

Biacore screening was used to select five CDR3-mutated Fabs based on off rate or K_D. CDR3-mutated Fabs were expressed in bacteria. Periplasmic extracts were prepared and screened in Biacore. The best clones were selected based on either off-rates or K_D for binding to hCTD or a peptide as shown in Table 4.

The biotinylated peptides or human CTD were coated on streptavidin chips. Periplasmic extracts from 10ml cultures were diluted ½ in HBS + 0.1% BSA. Samples were injected at 30µl/min for 3 minutes using the kinject program. Following a 3 minute dissociation, any remaining sample was stripped from chip surface. Off rates were measured on a time window of 1 min (between 10-70s). These data are presented in Tables 31 to 35.

On rates can be calculated from Biacore curves if both k_{off} and the Fab concentration are known. Under conditions of full Mass Transfer Limitation (MLT) like those encountered when working with very high density chips and low flow rates, the Biacore signal depends only on the concentration of the analyte run over the chip surface. Fab concentrations in crude samples can be determined from a

standard curve obtained by running different concentrations of a purified Fab on a high density Protein A chip. Using the Fab concentrations obtained that way and the k_{off} values, we calculated k_{on} data from the Biacore curves. The equilibrium dissociation constant K_D was obtained from k_{off}/k_{on}. These data are presented in Tables 31 to 35. Note that the 807B-M0079-D10 does not bind to Protein A. Therefore, only k_{off} values are presented for this clone.

Five variants were successfully selected for 807B-M0004-A03, 807B-M0004-H03, 807B-M0009-F06 and 807A-M0028-B02. The selected variants are identified in Table 36. No clones with significantly improved K_{off} were found for 807B-M0079-D10 (no K_D data available for this clone).

The selected Fabs were produced in *E. coli* and purified from periplasmic extracts by Immbolized Metal Affinity Chromatography. The quality of the preparations was checked on reducing and non reducing SDS-PAGE.

The purified Fabs were used to accurately determine the equilibrium dissociation constant K_D.

Biotinylated peptides or human CTD were coated on streptavidin chips. Experiments were performed in HBS running buffer. Purified Fabs were diluted to 200nM and serial ½ dilutions to 12.5nM were made and run in duplicates. For association, samples were injected at 30-40µl/min using the kinject program. Following a 3 minute dissociation, any remaining sample was stripped from the chip surface. Sensorgrams were analyzed using the simultaneous ka/kd fitting program in the BIAevaluation software 3.1. The data are summarized in Table 36. The best clones selected from Biacore screening exhibit an affinity 2 to 3-fold higher than the original clone for 807B-M0004-A03, 807B-M0009-F06 and 807A-M0028-B02. In the case of 807B-M0004-H03, the Biacore signals did not allow an accurate comparison of the different clones.

Purified Fabs were also tested by immunohistochemistry. Table 37 shows the names of the chosen clones together with the wild type clones and indicates whether they stain plaques in immunohistochemistry.

Example 39: Light chain shuffling of the antibody variants selected from Example 38

As a starting point for light chain shuffling (cycle 2 in Figure 29), the heavy chains corresponding to the VH-CDR3 improved variants from Example 38 (cycle 1 in Figure 29) were used together with wild-type (WT) clones.

The WT clone 807B-M0009-F06 was not included because the affinity of this clone was significantly lower compared to the selected variants from cycle 1. For antibody 807B-M0079-D10, as no improved affinity variant was found in cycle 1, the LC shuffling was performed on the WT clone alone.

In this Example, the non-affinity matured antibodies have been designated as the wild type (WT) clone and the selected variants from cycle 1 as parental clones.

The selected heavy chain variants from cycle 1 were cloned into the FAB310 vector backbone containing a repertoire of 5 to 6 heaving chains (HC) shuffled with approximately 10^8 light chains (LC) to create combinatorial diversity.

For every clone, a Qiagen DNA preparation was performed on a TG1 culture. 10μg of DNA was then cleaved using SfiI and NotI restriction enzymes, generating a heavy chain fragment size of 650 bp. The FAB310 vector backbone was similarly cut.

The resulting cleaved products (both vector and fragments) were gelpurified and, for each library, the different heavy chain variant fragments were pooled in equal amounts and 3μg of the pooled fragments were ligated with 6μg of cleaved phagemid vector backbone using T_4 DNA ligase (NEB). Desalted ligation mixtures for each library were introduced into *E. coli* TG1 cells by electroporation.

The library sizes achieved were such that each heavy chain variant was combined with at least one copy of each member of the light chain repertoire.

Heavy chain sequences were determined for 50 isolates, randomly picked from each library.

The light chain sequences of 48 isolates randomly picked from the 807A-M0028-B02-derived unselected library and 48 from the 807B-M0009-F06-derived unselected library. 63 unique functional light chains were obtained.

Phagemid particles were rescued with helper phage M13-KO7 (Marks *et al.* J. Mol. Biol. 222, 581 (1991)) using enough bacteria from each library for inoculation in order to represent each clone at least once.

The 5 light chained shuffled libraries were selected for improved affinity variants. Prior to selections the libraries were depleted for streptavidin binding antibodies by pre-incubating the libraries with 100µl streptavidin paramagnetic beads in 1ml 2% MPBS. For each library three concentrations of antigen were used to determine the optimal concentration for the second round of selection (Table 5). The incubation time of the phage with bead-target complex was reduced to 0.5 hour and 11 cycles of programmed washing was used in the Kingfisher device. After selection the bound phage were eluted and infected with *E. coli* (TG1 OD of 0.5) and the liquid amplified overnight at 30 ° C with shaking at 250 rpm in 25 ml 2xTY/Ampiclillin (100ug/ml) Glucose (2% w/v). Cells were concentrated and glycerol stocks were made in order to perform the round 2 selection.

Unselected library and output was titrated to get single colonies for picking and screening. From the unselected library and output library 47 colonies were picked and screened in a phage ELISA (Coated antigen 0.5 µg/ml for all antigens via b-BSA plus streptavidin).

All selection arms resulted in the enrichment of antigen binding clones. Based on these results an antigen concentration was chosen for the second round of selection.

The conditions for the round 2 selection were chosen to be more stringent and were designed to select for improved (faster) k_{on} and improved (slower) k_{off}. Three strategies were used as outlined in Table 6: Strategy I - further lowering of antigen concentrations; strategy II - further lowering of antigen concentration and reduced incubation time with antigen (k_{on} selection); strategy III - further lowering of antigen concentration and increased stringency washing (k_{off} selection). Selection was performed in a KingFisher automated device and an input of approximately 10^{12} phages were used. Prior to selections the libraries were depleted for streptavidin binding antibodies as described above. After selection the bound phage were eluted and used to infect *E. coli* as described previously. Cells were concentrated and glycerol stocks were made.

The output of round 2 was pre-screened in ELISA to determine the percentage of antigen binding clones. Sequence analysis of a limited number of clones was performed to determine if any particular clone was dominating selection and if there are any dominant light chain families in the selected clones.

Each of the round 2 phage outputs (15 in total) were re-cloned in batch in order to produce soluble Fabs. This was achieved through the removal of the geneIII stump from the vector.

200 clones were randomly picked and screened by ELISA for their binding to their respective antigen. Antibody sequences were determined for the positive hits only. The storage and initial sequence analysis was conducted via Dyax WEBPHAGE database.

For libraries 807B-M0004-A03, 807B-M079-D10 and 807A-M0004-H03, antibodies enriched between 2 to 8 times were selected. For libraries 807A-M0028-B02 and 807B-M009-F06, all clones were taken since number of unique clones was low. The light and heavy chain sequences from all ELISA positive and unique hits are shown in Tables 38 to 42.

After Biacore analysis of the potential affinity matured binders, a few were found to have a higher affinity. Only libraries 807B-M0004-A03, 807A-M0028-B02 and 807B-M004-H03 gave affinity matured antibodies with a higher affinity than the WT and parental clones. The light chain sequence of those selected clones was aligned to the germline. Interestingly, the same amino acid positions seem to be diversified among all the selected clones belonging to the same germline. The same amino acid positions seem to be diversified among all the selected clones belonging to the same germline.

For library 807B-M0004-A03, although clones M148E08 and M150E03 have exactly the same heavy chain sequence and light chains which differ by two amino acids, clone M150E03 has a 3.2x improved affinity compared to M148E08, suggesting that only two amino acids located in FR1 are responsible for this improvement.

For libraries 807B-M0028-B02 and 807B-M0004-A03, most of the diversification observed in the CDR and FR was reversion to the germline sequence compared to wild-type.

Only a few conservative variations were observed in FR3 of 807A-M0028 B02 library-derived clones.

For Biacore analysis, all clone variants were grown in small cultures (typically 10ml), periplasmic extracts (PE) were prepared and the Fab concentration in the PEs was measured by running the samples on a Protein A/G chip. The PEs

were then diluted to the same Fab concentration (25-50nM) and run over a target-coated chip (peptide 4, 8, 9 or CTD). The best clones were identified based either on the amplitude of the association and dissociation phases (807A-M0028-B02, 807B-M0004-A03, 807B-M0009-F06) or on the value of the Biacore signal at equilibrium (807B-M0004-H03). Clones derived from 807B-M0079-D10 do not bind to Protein A/G chips and were thus ranked only based on their off-rates

Clones derived from 807B-M0004-H03 were ranked based on the value of the Biacore signal at equilibrium which reflects the equilibrium dissociation constant K_D: the higher the Biacore signal at equilibrium, the better the affinity.

Clones derived from 807B-M0079-D10 were ranked based only on k_{off}.

Four and five variants were selected for 807A-M0028-B02 and 807B-M0004-A03, respectively. None of the isolated 807B-M0009-F06 variants seemed to exhibit an affinity higher than the best variants obtained from CDR3 spiking. For 807B-M0004-H03, two variants were selected based on the value of the Biacore signal at equilibrium. None of the 807B-M0079-D10 variants could be selected based on off-rate analysis.

The selected clones selected were produced in *E. coli* and purified from periplasmic extracts. This material was used to measure accurately the equilibrium dissociation constant K_D in Biacore. The data are summarized in Table 7. The best clones isolated from light chain shuffling exhibit an affinity ~ 5-times better than the corresponding original wild type clones or the best clones isolated from CDR3 spiking.

The clones selected following light chain shuffling are shown in Tables 43 and 44.

Table 1: Off-rate measurement of sFabs originating from selections on fibrils and ur-bCTD

Initial name	HCDR3	koff ($e^{-3}s^{-1}$)	RU
807A-M0027-C11	AVGYGDYGDY	13,30	79,2
807A-M0027-H05	DFFTSYFDH	16,90	182,0
807A-M0026-F11	DLWFGEWDY	28,10	165,8
807A-M0026-H09	DLWFGEWDY	25,10	138,0
807A-M0027-E12	DLWFGEWDY	8,95	419,9
807A-M0028-B12	DLWFGEWDY	18,80	335,5
807A-M0029-G10	DLWFGEWDY	23,80	212,0
807A-M0027-G01	DRGVSLLGAFDI	30,00	231,3
807A-M0028-A07	ESIAVAGVDY	53,60	367,0
807A-M0026-F05	GRGNYDFWSAGYYYYYMDV	>	158,0
807A-M0028-G07	QEVWQWPAQFDS	35,30	131,7
807A-M0027-E11	SLDLDY	40,50	412,3
807A-M0026-G08	SSGIYYGYYMDV	38,80	594,1
807A-M0028-B02	SVLLDY	28,00	454,6
807A-M0028-B06	DRGVSLLGAFDI	13,50	100,3
807A-M0027-D05	EPIWGYYYYGMDV	9,16	377,8

Table 2: Comparison of Fab and IgG binding on CTD-coated chip for the candidate clones 807A-M0026-F05 (26F5), 807A-M0027-E11 (27E11) and 807A-M0028-B02 (28B2)

Clone	Format	Surface	ka (1/Ms) e5	kd (1/s) e-3	KD (nM)
27E11	Fab	HD	9.5	45.5	47.8 ± 8.1
	IgG	HD	7.5	7.4*	9.9 ± 4.1
			8.1	2.1#	2.6 ± 1.1
		LD	10.1	7.7*	7.6 ± 3.2
			11.1	2.1#	1.9 ± 0.8
28B2	Fab	HD	2.5	44.2	179 ± 5.7
	IgG	HD	3.7	4.0	10.8 ± 2.4
		LD	5.2	6.4	12.3 ± 3.7
26F5	Fab	HD	n.f.	n.f.	n.d.
	IgG	HD	0.2	42	2103 ± 785
		LD	n.f.	n.f.	n.d.

* kd measured directly after injection stop.
# kd measured ~50 sec after injection stop.
n.f. no fit
n.d. not determined

Table 3

Antibody	Round 1 selection	Round 2 selection
807A-M0028-B02	3nM bCTD or 1 tube of fibrils	0.3nM bCTD + 5µM wt Fab or 0.3nM IgG1
807B-M0004-A03	5.7nM p4	0.057nM p4 + 5µM wt Fab or 0.01nM IgG1
807B-M0004-H03	5.7nM p4	0.57nM p4 + 5µM wt Fab or 0.3nM IgG1
807B-M0009-F06	5.7nM p9	0.057nM p9 + 5µM wt Fab or 0.3nM IgG1
807B-M0079-D10	5.7nM p8	0.057nM p8 + 5µM wt Fab or 0.3nM IgG1

Table 4

Original clone	*K_D (nM)*	*Number of clones screened*
807A-M0028-B02	150 (on hCDT)	72
807B-M0004-A03	98 (on p4)	39
807B-M0004-H03	200 (on p4)	54
807B-M0009-F06	172 (on p9)	24
807B-M0079-D10	26 (on p8)	33

Table 5: Percentage of antigen binding clones retrieved from the round 1 selections at decreasing antigen concentrations

Library	*Round*	*Antigen*	*Ag concentrations*
A03	1	P4	57nM, 5.7nM, 0.57nM
Hit rate% Input			2% 2% 2%
Output			62% 51% 83%
H03	1	P4	570nM, 57nM, 5.7nM
Hit rate% Input			2% 0% 0%
Output			72% 72% 81%
F06	1	P9	57nM, 5.7nM, 0.57nM
Hit rate% Input			0% 0% 0%
Output			6% 4% 2%
D10	1	P8	57nM, 5.7nM, 0.57nM
Hit rate% Input			0% 0% 0%
Output			34% 45% 38%
B2	1	CTD	300nM, 30nM, 3nM
Hit rate% Input			2% 2% 2%
Output			28% 21% 17%

Table 6: Conditions used for the second round selection strategies

Strategy	*Library*	*Round*	*Antigen*	*Ag conc (nM)*	*Incubation time (mins)*	*Washing*
I	A3	2	Pep-4	0.057	30	11x 5 mins
I	H3	2	Pep-4	5.7	30	11x 5 mins
I	D10	2	Pep-8	0.057	30	11x 5 mins
I	B2	2	b-CTD	10	30	11x 5 mins
I	F6	2	Pep-9	5.7	30	11x 5 mins
II	A3	2	Pep-4	0.57	3	11x 5 mins
II	H3	2	Pep-4	5.7	3	11x 5 mins
II	D10	2	Pep-8	0.57	3	11x 5 mins
II	B2	2	b-CTD	30	3	11x 5 mins
II	F6	2	Pep-9	5.7	3	11x 5 mins
III	A3	2	Pep-4	0.57	30	3 x 5 mins + 3 x 45 mins+3x5 mins
III	H3	2	Pep-4	5.7	30	3 x 5 mins + 3 x 45 mins+3x5 mins
III	D10	2	Pep-8	0.57	30	3 x 5 mins + 3 x 45 mins+3x5 mins
III	B2	2	b-CTD	30	30	3 x 5 mins + 3 x 45 mins+3x5 mins
III	F6	2	Pep-9	5.7	30	3 x 5 mins + 3 x 45 mins+3x5 mins

Table 7

Clone name	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)	
M0004A03 WT	4,19E+05	3,31E-02	79	*
M0004A03-M0148-E08	9,51E+05	4,85E-02	51	*
M0004A03-M0149-F02	6,51E+05	2,41E-02	37	*
M0004A03-M0149-G11	4,12E+05	1,73E-02	42	*
M0004A03-M0150-E03	6,08E+05	9,89E-03	16	*
M0004A03-M0151-D09	5,35E+05	1,61E-02	30	*
M0028B02-M0168-D10	6,97E+04	2,97E-03	43	*
M0028B02-M0169-F03	1,73E+05	1,78E-03	10	*
M0028B02-M0171-E03	1,02E+05	2,61E-03	26	*
M0028B02-M0171-G02	8,99E+04	4,55E-03	51	*

* Kinetic analysis, 1:1 model

Table 8: Description of SEQ ID NOS: 21-164 and 171-206

Antibody Name	VH-CDR1	VH-CDR2	VH-CDR3	VH	VL-CDR1	VL-CDR2	VL-CDR3	VL	Polynucleotide encoding VH	Polynucleotide encoding VL
807A-M0027-E11	SEQ ID NO: 21	SEQ ID NO: 22	SEQ ID NO: 23	SEQ ID NO: 39	SEQ ID NO: 30	SEQ ID NO: 31	SEQ ID NO: 32	SEQ ID NO: 42	SEQ ID NO: 174	SEQ ID NO: 173
807A-M0028-B02	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 26	SEQ ID NO: 40	SEQ ID NO: 33	SEQ ID NO: 34	SEQ ID NO: 35	SEQ ID NO: 43	SEQ ID NO: 176	SEQ ID NO: 175
807A-M0026-F05	SEQ ID NO: 27	SEQ ID NO: 28	SEQ ID NO: 29	SEQ ID NO: 41	SEQ ID NO: 36	SEQ ID NO: 37	SEQ ID NO: 38	SEQ ID NO: 44	SEQ ID NO: 172	SEQ ID NO: 171
807B-M0001-B07	SEQ ID NO: 45	SEQ ID NO: 46	SEQ ID NO: 47	SEQ ID NO: 135	SEQ ID NO: 90	SEQ ID NO: 91	SEQ ID NO: 92	SEQ ID NO: 150	SEQ ID NO: 178	SEQ ID NO: 177
807B-M0004-A03	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 50	SEQ ID NO: 136	SEQ ID NO: 93	SEQ ID NO: 94	SEQ ID NO: 95	SEQ ID NO: 151	SEQ ID NO: 180	SEQ ID NO: 179
807B-M0004-A05	SEQ ID NO: 51	SEQ ID NO: 52	SEQ ID NO: 53	SEQ ID NO: 137	SEQ ID NO: 96	SEQ ID NO: 97	SEQ ID NO: 98	SEQ ID NO: 152	SEQ ID NO: 182	SEQ ID NO: 181
807B-M0004-C04	SEQ ID NO: 54	SEQ ID NO: 55	SEQ ID NO: 56	SEQ ID NO: 138	SEQ ID NO: 99	SEQ ID NO: 100	SEQ ID NO: 101	SEQ ID NO: 153	SEQ ID NO: 184	SEQ ID NO: 183
807B-M0004-C05	SEQ ID NO: 57	SEQ ID NO: 58	SEQ ID NO: 59	SEQ ID NO: 139	SEQ ID NO: 102	SEQ ID NO: 103	SEQ ID NO: 104	SEQ ID NO: 154	SEQ ID NO: 186	SEQ ID NO: 185
807B-M0004-F06	SEQ ID NO: 60	SEQ ID NO: 61	SEQ ID NO: 62	SEQ ID NO: 140	SEQ ID NO: 105	SEQ ID NO: 106	SEQ ID NO: 107	SEQ ID NO: 155	SEQ ID NO: 188	SEQ ID NO: 187
807B-M0004-F10	SEQ ID NO: 63	SEQ ID NO: 64	SEQ ID NO: 65	SEQ ID NO: 141	SEQ ID NO: 108	SEQ ID NO: 109	SEQ ID NO: 110	SEQ ID NO: 156	SEQ ID NO: 190	SEQ ID NO: 189
807B-M0004-H03	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 68	SEQ ID NO: 142	SEQ ID NO: 111	SEQ ID NO: 112	SEQ ID NO: 113	SEQ ID NO: 157	SEQ ID NO: 192	SEQ ID NO: 191
807B-M0009-C03	SEQ ID NO: 69	SEQ ID NO: 70	SEQ ID NO: 71	SEQ ID NO: 143	SEQ ID NO: 114	SEQ ID NO: 115	SEQ ID NO: 116	SEQ ID NO: 158	SEQ ID NO: 194	SEQ ID NO: 193
807B-M0009-F06	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 74	SEQ ID NO: 144	SEQ ID NO: 117	SEQ ID NO: 118	SEQ ID NO: 119	SEQ ID NO: 159	SEQ ID NO: 196	SEQ ID NO: 195
807B-M0013-A12	SEQ ID NO: 75	SEQ ID NO: 76	SEQ ID NO: 77	SEQ ID NO: 145	SEQ ID NO: 120	SEQ ID NO: 121	SEQ ID NO: 122	SEQ ID NO: 160	SEQ ID NO: 198	SEQ ID NO: 197
807B-M0079-D10	SEQ ID NO: 78	SEQ ID NO: 79	SEQ ID NO: 80	SEQ ID NO: 146	SEQ ID NO: 123	SEQ ID NO: 124	SEQ ID NO: 125	SEQ ID NO: 161	SEQ ID NO: 200	SEQ ID NO: 199
807B-M0081-F12	SEQ ID NO: 81	SEQ ID NO: 82	SEQ ID NO: 83	SEQ ID NO: 147	SEQ ID NO: 126	SEQ ID NO: 127	SEQ ID NO: 128	SEQ ID NO: 162	SEQ ID NO: 206	SEQ ID NO: 205
807B-M0081-H03	SEQ ID NO: 84	SEQ ID NO: 85	SEQ ID NO: 86	SEQ ID NO: 148	SEQ ID NO: 129	SEQ ID NO: 130	SEQ ID NO: 131	SEQ ID NO: 163	SEQ ID NO: 202	SEQ ID NO: 201
807B-M0083-E11	SEQ ID NO: 87	SEQ ID NO: 88	SEQ ID NO: 89	SEQ ID NO: 149	SEQ ID NO: 132	SEQ ID NO: 133	SEQ ID NO: 134	SEQ ID NO: 164	SEQ ID NO: 204	SEQ ID NO: 203

Table 9: Amino acid sequences of the VL chains of the antibodies identified using the screening strategy of Example 5

Initial Name	LV-FR1	LV-CDR1	LV-FR2	LV-CDR2	LV-FR3	LV-CDR3	LV-FR4
807A-M0027-C11	QDIQMTQSPSTLSASVGDRVTITC	RASQSVSSWLA	WYQQKPGAAPRLLIY	KASSLQT	GVPSRFSGGGSGTEFTLTISSLQPDDFATYYC	QQSYSTPT	FGGGTKVEIK
807A-M0043-F08	QDIQMTQSPSSVSASVGDRVTITC	RASQGISSWLA	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQANSFPPT	FGQGTKVEIQ
807A-M0039-E07	QSELTQPPSASGSPGQSVTISC	TGTSSDVGGYNYVS	WYQQHPGKAPKLMIY	EVSKRPS	GVPDRFSGSKSVTSASLAITGLQAEDEADYYC	QSYDSSLSGYV	FGSGTKVTVL
807A-M0039-D11	QDIQMTQSPSSLSASVGDRVTMTC	QASQDIRNYIN	WYQQKPGKAPKLLIN	DASNLEP	GVPSRFRGSGYGTDFSFSISSLQSEDIATYYC	QQYDSVPIT	FGQGTRVEIK
807A-M0037-F10	QSALTQPPSASGTPGQRVTISC	SGRSSNIGSNSVN	WYQQLPGTAPKLLIY	SNNQRPS	GVPDRFSGSKSGTSASLAISGLRSEDEADYYC	AAWDDSLSGVV	FGGGTKLTVL
807A-M0028-B06	QDIQMTQSPDTLSLSPGDRATLSC	RASQSVSSNYLA	WYHQKPGQAPRLVIY	NTSRRAT	GIPDRFSGSGSGTDFTLTISRLDPEDFGVYYC	QQYAYGRSPGYP	FGQGTRLEIK
807A-M0046-F05	QDIQMTQSPGTLSLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQTPRLLIY	GASSRAT	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQYGSSPYT	FGQGTKLEIR
807A-M0041-F03	QDIQMTQSPSTLSASVGDRVAITC	RASQGINRWLA	WYQQKPGKAPKLLIY	KASALES	GVPSRFSGSVSGTQFTLTISSLQPDDFATYYC	QHYYTYPYA	FGQGTKLEIK
807A-M0043-E08	QYELTQPPSASGSPGQSVTISC	TGTSSDVGAYNYVS	WYQQHPGKAPKLIIY	EVNKRPS	GVPDRFSASKSGNTASLTVSGLQAEDEADYYC	NSYAGSNSLI	FGGGTKLTVL
807A-M0042-D05	QDIQMTQSPATLSVSPGDRVTLSC	RASQSVGSTLA	WYQQKPGQAPRLLIY	GAVTRAT	GVPARFSASASGPDFTLTISSLQSEDFAVYYC	QQYGGSPWYT	FGQGTKLEIK
807A-M0029-G10	QDIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLIISDLQPEDFATYYC	QQSYTTPFT	FGPGTTVDIK
807A-M0046-G03	QDIQMTQSPSSLSASVGDRVTITC	RASQGITNWVA	WYQQKPGKAPKLLIY	GASRLQS	GVPSRFSGSGSGTDFSLTISSLQPEDFATYYC	QQSYSSLFT	FGPGTKVDIK
807A-M0037-D06	QDIQMTQSPSSLSASVGDRVTITC	RTSQDVRNWVA	WYQQKPGKAPNLLIY	MASTLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYFC	QQADTFPWT	FGQGTKVDIK
807A-M0043-E07	QDIQMTQSPSSLSASVGDRVTITC	RASQNVNTFLN	WYQHKAGKAPKLLIY	AASSLQS	GVPSRFSGTGSGTDFTLTISSLQPEDFATYYC	QQSYSDPLT	FGGGTKVEIK
807A-M0027-E11	QDIQMTQSPSSLSASVGDRVTITC	RASQRIRKNLH	WYQQKPGKAPNLLIY	DASSNER	GVPSRFSGRGSGTEFTLTISSLQPEDLATYYC	QQSFSSPWT	FGQGTKVEIK
807A-M0046-A11	QDIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTPLT	FGGGTKVEIK
807A-M0041-E01	QDIQMTQSPSSLSASVGDRVTITC	HASQDIANYLS	WYQQKPGKAPKLLIY	DAFNLET	GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC	QQFEDLFSLT	FGPGTRVDLK
807A-M0044-B07	QDIQMTQSPGILSLSPGERATLSC	RASQNLIFNFLA	WYQHKPGQAPRLLIY	GSSTRAT	GIPDRFSGSGSGTDFTLTINRLEPEDFAVYYC	QQYHTSSFT	FGPGTKVDIK
807A-M0028-B02	QDIQMTQSPSSLSASVGDRVTITC	RTSQDIRNHLG	WFQQKPGKAPQRLIR	EASILQS	GVPSTFYGSGYGREFTLTISSLQPEDFATYYC	LQYDSFPYT	FGQGTKLEIK
807A-M0039-E06	QDIQMTQSPSSVSASVGDRVTITC	RASQGISSWLA	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFASYFC	QQSYSSPGIT	FGPGTKVEIK

807A-M0040-A03	QSELTQPPSVSVSPGQTA TITC	SGDKLGDKYAS	WYQQRPGQSPVLVIY	QDTKRPS	GIPERFSGSNSGNTATLTISGTQT MDEADYYC	QAWGSSPVV	FGGGTRLTVL
807A-M0044-G07	QDIQMTQSPSSVSASVGD RVTITC	RASQVISTWLS	WYQQKPGKAPKLLIY	TASTLQS	GVPARFSGSGSGTDFTLTINNLQP EDFATYYC	QQANSFPIT	FGGGTKVEIN
807A-M0044-E08	QSPSSVSTSVGDRVTITCR ASQ	LDIQMTDISTWLA	WYQQKPGKAPKLLIY	AASTLES	GVPSRFSGSGSGTDFTLTISSLQP EDFATYYC	QQAYSFPIT	FGQGTRLEIK
807A-M0038-A09	QSALTQPPSVSVAPGQTA RITC	GGNNIGTKIVN	WYQQRPGQAPVVVVY	DNSDRPS	GIPERFSGSNSGNTATLTISRVEA GDEADYYC	QLWDSSSDHPI	FGTGTKVTVL
807A-M0037-C08	QDIQMTQSPSSVSASVGD RVTITC	RASQGISSWLA	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSASGADFTLTISSLQP EDFATYYC	QQTYDTPFT	FGPGTTVDLK
807A-M0039-H09	QDIQMTQSPSSLPASVGD SVTVTC	RTSQSISDYVN	WYQQKPGKAPNLLIY	AASTLQG	GVPSRFSGSASGTNFSLTIDDLQP EDFATYYC	QQTFFSPPT	FGQGTRVEIK
807A-M0039-D05	QDIQMTQSPSSLSASVGD RVTITC	RASQDIRDDLG	WYQQKPGKAPKRLIY	AASSLQS	GVPSRFSGSGSGTEFTLTISSLQP EDFATYYC	QQHNNYPSFT	FGPGTRLDIK
807A-M0042-F12	QSELTQPPSASGTPGQRV TISC	SGGYSNMGSNYAH	WYQQLPGTAPKLLIY	NNNQRPS	GVPDRFSGSKSGTSASLAISGLQS EDEADYYC	AAWDDSLNGWV	FGGGTKLTVL
807A-M0043-H05	QSELTQPASVSGSPGQSIT ISC	TGTNTDVGGYNYVA	WYQQHPGKAPKLMIY	DVSNRPS	GVSTRFSGSKSGDTASLTISGLQT EDEADYYC	SSFTSRSTHV	FGTGTKVTVL
807A-M0042-C03	QDIQMTQSPATLSLSPGER ATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDRFSGSGSGTDFTLTISRLEPE DFASYFC	QQSYSSPGIT	FGPGTKVEIK
807A-M0040-C03	QDIQMTQSPGTLSLSPGER ATLSC	RASQIFSSSYVA	WYQQKPGQAPRLLIY	GASSRAS	GIPDRFSGSGSGTDFTLTISRLEPE DFAVYWC	QQSSSSPPT	FGQGTRVEVR
807A-M0046-C05	QDIQMTQSPSSLSASVGD RVTITC	RASQSISSYLN	WYQQKPGKAPKVLIY	GTSSLQS	GVPSRFSGSGSRTDFTLTISSLQP EDFGIYYC	QQSYNTPPT	FGQGTKLEIK
807A-M0027-D05	QDIQMTQSPSSLSASVGD RVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQP EDFATYYC	QQTYTTPAWT	FGQGTKVEIR
807A-M0040-B11	QSVLTQPPSASGTPGQRV TISC	SGSSSNIGSNNVN	WYQQLPGTAPKLLIY	SNDQRPS	GVPDRFSGSKSATSASLAISGLQS EDEADYHC	AAWDDSLNGPV	FGGGTKLTVL
807A-M0039-B02	QDIQMTQSPSTLSASVGD RVTITC	RASQSISSWLA	WYQQKPGKAPKLLIY	TASSLES	GVPSRFSAGGSGTEFTLTISSLQP DDFGTYYC	QQYNSYSLT	FGGGTKVEIK
807A-M0041-C07	QDIQMTQSPSSLSASLGDR VTITC	RASQGISNSLA	WYQQKPGKAPKLLIS	AASTLQT	GVPSRFSGSGSGTDFTLIITNLQPD DFATYYC	QQINGYPVT	FGAGTKVEIK
807A-M0041-H04	QSELTQPPSASGTPGQRVI ISC	SGSGSNIGSNIVS	WFQQLPGAAPRLLIY	NDHRRPS	GVPDRFSGSKSGTSASLAITGLRS EDETDYYC	AAWDDSLSAVV	FGGGTKLTVL
807A-M0028-G07	QYELTQPPSVSVAPGQTA RITC	GGNNIGSKNVH	WYQQKPGQAPVLVVY	DDTDRPS	GIPERFSGSNSGDTATLTISWVEA GDEAKYHC	HVWDSSSDHYV	FGTGTAVTVL
807A-M0041-A09	QDIQMTQSPATLSLSPGER ATLSC	RASQSVSNNLA	WYQQKPGQAPRLLIS	GASTRAT	GIPARFSGSGSGTEFTLTINSLQSE DSAVYYC	QQYDNWPPFT	FGPGTKVDIK
807A-M0042-B10	QSALTQPASVSGSPGQSIT ISC	SGTDSDVGGYNHVS	WYQQHPGKAPKLIIY	DVDHRPS	GISNRFSGSKSGNTASLTISGLQA EDEADYYC	SSYRSGSTYV	FGTATKVTVL
807A-M0041-E06	QSVLTQPPSTSGTPGQRV TISC	SGSNSNIGSKTVN	WYQQLPGTAPKLLIY	MNYERPS	GVPDRFSGSKSGTSASLAISGLQS EDEADYYC	AAWDDSLSGPV	FGGGTKLTVL
807A-M0037-D10	QDIQMTQSPSSLSASVGD RVTITC	RASQSIYTSLN	WYQQKPGKAPRLLIS	DASNLQS	GVPSRFSGSGSGTDFTLTIASLQP DDFATYHC	QQSYRLFPT	FGQGTRLEIK

807A-M0044-F04	QDIQMTQSPATLSVSPGGRATLSC	RASQSVRKNVA	WYQQKPGQPPRLLIY	GASTRAT	GVPARFSGSGSGTEFTLTISRMQPEDFVVYHC	QQYSSWPA	FGQGTMVEIN
807A-M0043-D10	QDIQMTQSPATLSVSPGEGATLSC	RASQSVSSGLA	WYQQKPGQSPRLLIY	DISTRAT	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC	QQYKDWPLT	FGGGTQVEIK
807A-M0043-G06	QDIQMTQSPSYLSASVGDRVTITC	RASQTISRYLN	WYQQKPGNAPKLLIY	AASTLQS	GVPSRFSGSGSGTDFTLAISSLQPEDFATYYC	QNSYSSPYT	FGQGTNVELK
807A-M0037-G01	QDIQMTQSPATLSVSPGERATLSC	RASQSVSSNLA	WYQQKPGQAPRLLIY	GASTRAT	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC	QQYGSSPPIT	FGQGTRLEIK
807A-M0044-E11	QSALTQPASVSGSPGQSITISC	TGTSTDVGGYNYVS	WYQKHPGKAPKLMIY	DVSNRPS	GVSNRFSGSKSGNTASLTISGLQAEDEADYYC	SSYTNTITVV	FGGGTKLTVL
807A-M0043-A10	QDIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	TTSFVQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYTIPTT	FGGGTKVDVK
807A-M0045-B03	QYELTQPASVSGSPGQSITISC	TGTSSDVGAFNYVS	WYQHHPGKAPKLLLY	EVTNRPS	GVSDRFSGSKSGNTASLTISGLQAEDEADYHC	ASYTRTRSLA	FGGGTRLTVL
807A-M0038-A08	QDIQMTQSPSSLSASVGDRVTITC	RASQSIRIYLN	WYQQKPGKAPKLLIY	AASKLED	GVPSRFSGSGTGTDFTLTIRSLQPEDFASYFC	QQSYSSPGIT	FGPGTKVEIK
807A-M0039-C02	QDIQMTQSPGTLSLSPGDRATLSC	RASQSVGSDYLA	WYQQKPGQAPRLLIF	AASTRAT	GIPDRFSGSGSATDFTLTISSLEPEDFAVYFC	QQYASPPRT	FGQGTKVEIK
807A-M0027-G01	QSELTQPPSASGTPGQRVTISC	SGGYSNMGSNYAH	WYQQLPGTAPKLLIY	NNNQRPS	GVPDRFSGSKSGTSASLAISGLRSEDEADYYC	AAWDDSLSGPV	FGGGTKLTVL
807A-M0039-B08	QDIQMTQSPSSLSASVGDRVTITC	RASQGISNFLA	WYQQKPGKAPKVLIY	DASTLRS	GVPSRFSGSGSGTDFTLTIDSLQPEDFATYYC	QQYYRYPLT	FGGGTKVEIK
807A-M0046-E12	QDIQMTQSPSSLSASVGDRVTITC	RASQGIRNDLG	WYQQKPGKAPKRLIY	GASSLQS	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	LQHNSYPLT	FGGGTKVEIK
807A-M0041-A08	QSELTQPASVSGSPGQSITISC	TGTSSDVGGYNYVS	WYQQHPGKAPKLMIY	DVSNRPS	GVSNRFSGSKSGNTASLTISGLQAEDEADYYC	SSYTSSSTLDPYA	FGTGTKVTVL
807A-M0037-C09	QDIQMTQSPSSLSASVGDRVTVTC	RASQGIRNNLA	WYQQRPGKAPKRLIY	GASNLHS	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	LQHNNYPYS	FGQGTKLEIK
807A-M0040-G01	QDIQMTQSPSSLSASVGDRVTITC	RASQGIHNYVN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSATDFTLTISSLQPEDFATYYC	QQSFNTPFT	FGPGTRVDIK
807A-M0045-E04	QDIQMTQSPSSLSASVGDRVTITC	RASQGIRKDLG	WYQQRPGKAPKLLIY	GASSLLN	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	LQDNDYPFT	FGPGTKVEIR
807A-M0041-H05	QSALTQPPSVSGAPGQRVTISC	TGSSSNIGAPYDVH	WYQQVPGTAPKVLIY	GNNHRPS	GVPDRFSGSKSGTSASLAISGLQAEDEAHYYC	QSYDSSLSGPI	FGGGTTLTVL
807A-M0043-A08	QSALTQPASVSGSPGQSITISC	TGTSNDVGGYNSVS	WYQQHPGKAPKLLIY	DVTNRPS	GVSNRFSTSQSANTASLTISGLQPEDEAEYFC	SSYTTRSTWV	FGGGTKLTVL
807A-M0038-C09	QDIQMTQSPDSLAVSLGERATINC	KSSQSVLYSSNNKNYLA	WYQQKPGQPPKLLIY	WASIRES	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	QQYYSTPTWT	FGQGTKVEIK
807A-M0042-F09	QDIQMTQSPSSLSASVGDRVTITC	RASQGIRHDLG	WYQQKPGKAPKRLIY	AASSLQN	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	LQHNSYPWT	FGQGTKVEIK
807A-M0045-B12	QDIQMTQSPSSLSASVGDRVTITC	RTSQNINTYLN	WYQQKPGKAPRLLIY	AASSLQS	GVPSRFSGSGFGTDFTLTISSLQPEDFGIYYC	EQSYNVPRT	FGQGTRLDIK
807A-M0044-C04	QDIQMTQSPSSLSASVGDRVTITC	RASQTISNYLN	WYQQKPGKAPKLLIY	ATSTLQS	GVPSRFSGSGSGTDFTLTITSLQPEDFATYYC	QQTYNTPGT	FGQGTKLEIK

807A-M0026-F11	QSVLTQPASVSGSPGQSITISC	TGTSSDVGIYNYVS	WYQQHPGKAPKLMIY	DVSNRPS	GVSNRFSGSKSGNTASLTISGLQAEDEADYYC	SSYTSSSTPYV	FGTGTKVTVL
807A-M0027-E12	QDIQMTQSPGTLSLSPGERATLSC	RASRSLFSTYLA	WYQQKPGQPPRLLIY	GASTRAT	GIPDRFSGSGSGTDFTLTISRLEPEDSALYYC	QQYVSSQLT	FGGGTKVEIK
807A-M0028-B12	QDIQMTQSPLSLSASAGDRVTITC	RASQNINRYLN	WYQQKPGKAPRLLIY	AASNLQS	GVPSRFSGSQSGTDFTLTISSLQPEDFATYYC	QQSFSPPIT	FGQGTRLDIK
807A-M0026-G08	QDIQMTQSPSSLSASAGDRVTITC	RANQGIRNNLA	WFQQKPGKAPKSLIY	DASSLQS	GVPSKFSGTGSGTEFTLTIGSLQPEDSATYYC	QQYFTFPLT	FGGGTKVEIK
807A-M0043-A07	QDIQMTQSPSSVSASVGDRVTITC	RASQGISSWLA	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQANSFPLT	FGGGTKVEIK
807A-M0042-F04	QDIQMTQSPATLSLSPGESATLSC	RASQSVNDYLA	WYQQKPGQAPRLLIY	DSSNRAT	GIPARFSGSGSGTDFTLTISSLEPEDFATYYC	QQANSFPPT	FGQGTKVEIK
807A-M0045-H09	QDIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	VASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSIPPT	FGQGTRVEIK
807A-M0046-D04	QDIQMTQSPGTLSLSPGESATLSC	RASQSISSYLN	WYQQKQGKAPKLLMF	AASSLKS	GIPDRFSGRGSGTDFSLTISRLEPEDFAVYYC	QQYEFSPEN	FGQGTKLQIK
807A-M0040-G04	QDIQMTQSPATLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTPHT	FGQGTKLEIK
807A-M0045-B01	QSVLTQPASVSGSPGQSITISC	TGTNTDVGGYNYVS	WYQQNPGEAPKLIIY	EVNHRPS	GVSDRFSGSKSGNTASLTISGLQADDETDYYC	SSYTNRNGYV	FGTGTKVTVL
807A-M0040-A08	QSVLTQPPSASGTPGQRVIISC	SGSSSNIGSNIVS	WFQQVPGAAPRLLIY	NDHRRPS	GVPDRFSGSKSGTSASLAISGLQSEDDADYYC	ASWDDSLNGVL	FGGGTKLTVL
807A-M0026-F05	QDIQMTQSPGTLSLSPGERATLSC	RASQSIGSRYLA	WYQQKPGQAPRLLIY	DASKRAT	GVPVRFSGSGSGTDFTLTISSLGPEDFAVYYC	QQGYNWPPWT	FGQGTKVEIK
807A-M0037-H02	QDIQMTQSPGTLSLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPSLLIY	DMSTRAP	GIPERFSGSGSGTDFTLTISRLEPEDFAVYYC	QQYGSSVA	FGGGTKVEMK
807A-M0042-A06	QSALTQPPSASGTPGQRVTISC	SGGYSNMGSNYAH	WYQQLPGTAPKLLIY	NNNQRPS	GVPDRFSGSKSGTSASLAISGLRSEDEADYYC	AAWDDSLSGPV	FGGGTKLTVL
807A-M0028-A07	QDIQMTQSPSSLSASIGDRVTITC	RASQGISNYLA	WYQQKPGKVPNLLIY	AASTLQS	GVPSRFSGSGSGTDFTLTISSLQPEDVATYYC	QKYNSAPRT	FGQGTKVEIK
807A-M0043-G01	QDIQMTQSPSTLSASVGDRVTITC	RPSQSTSNWLA	WYQQKPGKAPKLLIY	KASILES	GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC	QQYDSYWT	FGQGTKIEIK
807A-M0046-F04	QDIQMTQSPSSLSASVGDRVTITC	RASQSISTYLN	WYQHKPGNAPNLLIY	GASSLKR	GVPSRFSGSGSETEFTLTISSLQPEDFATYYC	QQSYSAPLI	FGGGTKVEIR
807A-M0037-F03	QDIQMTQSPGTLSLSPGERATLSC	RASQSISSRYLA	WYQQKAGQAPRLLMY	GASRAT	GIPARFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYEYPLT	FGQGTKLEIK
807A-M0039-C10	QSALTQPRSVSGSPGQSVTISC	TGTYSDVGNYYSVS	WYQQHPGKAPKFIIY	DVTKRPS	GVPDRFSGSKSGNTASLTISGLQAEDEADYYC	CSYAGSYTLL	FGGGTKLTVL

Table 10: Amino acid sequences of the VH chains of the antibodies identified using the screening strategy of Example 5

Initial Name	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
807A-M0027-C11	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	EYGMS	WVRQAPGKGLEWVS	VISPSGGGTEYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	AVGYGDYGDY	WGQGTLVTVSS
807A-M0043-F08	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	EYTML	WVRQAPGKGLEWVS	GIWPSGGPTFYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	ELDTAMAPPSDAFDI	WGQGTMVTVSS
807A-M0039-E07	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	FYFMG	WVRQAPGKGLEWVS	SISSSGGMTTYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0039-D11	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	FYGMG	WVRQAPGKGLEWVS	YISPSGGSTTYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0037-F10	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	FYGMG	WVRQAPGKGLEWVS	YISSSGGLTFYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0028-B06	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	FYQMN	WVRQAPGKGLEWVS	SIYPSGGLTYYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DRGVSLLGAFDI	WGQGTMVTVSS
807A-M0046-F05	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	FYRMT	WVRQAPGKGLEWVS	SISSSGGGTPYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0041-F03	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	FYSMF	WVRQAPGKGLEWVS	YIYPSGGWTNYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	SVVGWGLDY	WGQGTLVTVSS
807A-M0043-E08	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	FYSMG	WVRQAPGKGLEWVS	YIYPSGGGTTYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0042-D05	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	FYSMV	WVRQAPGKGLEWVS	SISPSGGQTDYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0029-G10	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	FYTMV	WVRQAPGKGLEWVS	VISPSGGLTHYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0046-G03	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	FYYMS	WVRQAPGKGLEWVS	RISPSGGLTHYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DFFTSYFDY	WGQGTLVTVSS
807A-M0037-D06	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	HYLMV	WVRQAPGKGLEWVS	GISPSGGGTNYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAK	GPGYSYGYYYYGMDV	WGQGTTVTVSS
807A-M0043-E07	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	KYPMQ	WVRQAPGKGLEWVS	SISPSGGSTVYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	GVTTVPRYYYYYYMDV	WGKGTTVTVSS
807A-M0027-E11	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	KYSMH	WVRQAPGKGLEWVS	GIYSSGGKTIYADSVKG	RFTISRDNPKNTLYLQMNS LRAEDTAVYYCAR	SLDLDY	WGQGTLVTVSS
807A-M0046-A11	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	LYGMV	WVRQAPGKGLEWVS	RISPSGGYTGYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0041-E01	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	LYRMG	WVRQAPGKGLEWVS	SISPSGGWTRYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0044-B07	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	MYGML	WVRQAPGKGLEWVS	RISPSGGFTNYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0028-B02	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	MYMMD	WVRQAPGKGLEWVS	SIWPSGGQTWYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	SVLLDY	WGQGTLVTVSS
807A-M0039-E06	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	NYVMH	WVRQAPGKGLEWVS	VISPSGGATIYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DWGPFEAFDI	WGQGTMVTVSS
807A-M0040-A03	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	QYNMG	WVRQAPGKGLEWVS	YISSSGGITWYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DFFTSYFDY	WGQGTLVTVSS

807A-M0044-G07	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	TYSMH	WVRQAPGKGLEWVS	YIGSSGGFTMYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	GLYR	WGQGTLVTVSS
807A-M0044-E08	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	TYWMI	WVRQAPGKGLEWVS	SISSSGGWTMYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EDNYYGMDV	WGQGTTVTVSS
807A-M0038-A09	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	VYSMA	WVRQAPGKGLEWVS	GIWPSGGPTAYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EDFWSGLEDV	WGKGTTVTVSS
807A-M0037-C08	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYAMD	WVRQAPGKGLEWVS	RIRPSGGNTDYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0039-H09	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYAMD	WVRQAPGKGLEWVS	RIRSSGGLTHYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0039-D05	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYAMD	WVRQAPGKGLEWVS	SIYPSGGWTEYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	GLGMDV	WGQGTTVTVSS
807A-M0042-F12	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYAMS	WVRQAPGKGLEWVS	SIYSSGGKTGYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0043-H05	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYAMV	WVRQAPGKGLEWVS	YIRSSGGHTVYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0046-H06	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMD	WVRQAPGKGLEWVS	SISSSGGFTTYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0042-C03	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMD	WVRQAPGKGLEWVS	SIVSSGGLTDYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	QEVWQWPAQFDS	WGQGTLVTVSS
807A-M0040-C03	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDME	WVRQAPGKGLEWVS	VIGPSGGPTHYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0046-C05	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDME	WVRQAPGKGLEWVS	WISSSGGTTWYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0027-D05	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMF	WVRQAPGKGLEWVS	SIYSSGGITYYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0040-B11	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMG	WVRQAPGKGLEWVS	SISPSGGSTTYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0039-B02	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMH	WVRQAPGKGLEWVS	SISPSGGLTYYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	QEVWQWPAQFDS	WGQGTLVTVSS
807A-M0041-C07	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMH	WVRQAPGKGLEWVS	SISPSGGLTYYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	QEVWQWPAQFDS	WGQGTLVTVSS
807A-M0041-H04	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMH	WVRQAPGKGLEWVS	SISSSGGDTTYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0028-G07	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMH	WVRQAPGKGLEWVS	YISPSGGWTGYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	QEVWQWPAQFDS	WGQGTLVTVSS
807A-M0041-A09	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMN	WVRQAPGKGLEWVS	RISPSGGSTRYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0042-B10	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMN	WVRQAPGKGLEWVS	SIGSSGGETRYADSVKG	RFTISRDNSKNTLYLQMNS LKAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0041-E06	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMQ	WVRQAPGKGLEWVS	SISSSGGLTTYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0037-D10	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMR	WVRQAPGKGLEWVS	SISSSGGRTVYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS

807A-M0044-F04	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMS	WVRQAPGKGLEWVS	RIGSSGGATQYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0043-D10	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMS	WVRQAPGKGLEWVS	YIVPSGGVTLYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0043-G06	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMV	WVRQAPGKGLEWVS	SIVSSGGETRYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0037-G01	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMV	WVRQAPGKGLEWVS	YIRPSGGITFYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	QEVWQWPAQFDS	WGQGTLVTVSS
807A-M0044-E11	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMV	WVRQAPGKGLEWVS	YIVPSGGETSYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0043-A10	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMY	WVRQAPGKGLEWVS	GIGPSGGSTYYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0045-B03	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMY	WVRQAPGKGLEWVS	SIRSSGGLTNYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTLVTVSS
807A-M0038-A08	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMY	WVRQAPGKGLEWVS	SISPSGGITAYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	QEVWQWPAQFDS	WGQGTLVTVSS
807A-M0039-C02	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYDMY	WVRQAPGKGLEWVS	SISPSGGITAYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	QEVWQWPAQFDS	WGQGTLVTVSS
807A-M0027-G01	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYEMG	WVRQAPGKGLEWVS	SIGPSGGETIYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DRGVSLLGAFDI	WGQGTMVTVSS
807A-M0039-B08	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYEMG	WVRQAPGKGLEWVS	SIYSSGGQTVYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	QEVWQWPAQFDS	WGQGTLVTVSS
807A-M0046-E12	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYEMN	WVRQAPGKGLEWVS	RIYPSGGPTWYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0041-A08	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYFMH	WVRQAPGKGLEWVS	SIYPSGGTTEYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0037-C09	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYGMG	WVRQAPGKGLEWVS	YISSSGGLTIYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0040-G01	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYGMS	WVRQAPGKGLEWVS	RIVSSGGPTGYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0045-E04	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYGMV	WVRQAPGKGLEWVS	SIYPSGGTTQYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0041-H05	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYNMD	WVRQAPGKGLEWVS	SIGPSGGPTKYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0043-A08	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYNMG	WVRQAPGKGLEWVS	YIGPSGGETYYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0038-C09	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYNMQ	WVRQAPGKGLEWVS	VISPSGGGTYYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	VKWDHSPLFDP	WGQGTLVTVSS
807A-M0042-F09	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYNMV	WVRQAPGKGLEWVS	WISSSGGMTRYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0045-B12	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYPMY	WVRQAPGKGLEWVS	VIYPSGGHTKYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0044-C04	EVQLLESGGGLVQPGG SLRLSCAASGFTFS	WYQME	WVRQAPGKGLEWVS	SISSSGGTTDYADSVKG	RFTISRDNSKNTLYLQMNS LRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS

807A-M0026-F11	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYQMS	WVRQAPGKGLEWVS	SISSSGGHTFYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0027-E12	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYRMT	WVRQAPGKGLEWVS	SISPSGGVTLYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0028-B12	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYSMG	WVRQAPGKGLEWVS	WISSSGGGTPYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0026-G08	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYSMH	WVRQAPGKGLEWVS	SIRSSGGWTKYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	SSGIYYGYYMDV	WGKGATVTVSS
807A-M0043-A07	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYSML	WVRQAPGKGLEWVS	YIYPSGGATFYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GRSTFDI	WGQGTMVTVSS
807A-M0042-F04	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYSMN	WVRQAPGKGLEWVS	GISSSGGMTHYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0045-H09	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYTMG	WVRQAPGKGLEWVS	SIVSSGGYTPYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0046-D04	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYTMH	WVRQAPGKGLEWVS	SIRSSGGMTDYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	SSGIYYGYYMDV	WGKGATVTVSS
807A-M0040-G04	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYTMQ	WVRQAPGKGLEWVS	SIYPSGGDTKYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0045-B01	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYTMV	WVRQAPGKGLEWVS	SIRSSGGQTKYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0040-A08	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYTMV	WVRQAPGKGLEWVS	SIVPSGGDTHYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807A-M0026-F05	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	YYAMQ	WVRQAPGKGLEWVS	SLYPSGGNTSYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GRGNYDFWSAGYYYYYMDV	WGKGTTVTVSS
807A-M0037-H02	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	YYGMV	WVRQAPGKGLEWVS	RISPSGGMTDYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTPVTVSS
807A-M0042-A06	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	YYHMD	WVRQAPGKGLEWVS	SIVSSGGFTAYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DRGVSLLGAFDI	WGQGTMVTVSS
807A-M0028-A07	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	YYRMA	WVRQAPGKGLEWVS	SIYSSGGMTLYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	ESIAVAGVDY	WGQGTLVTVSS
807A-M0043-G01	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	YYSMI	WVRQAPGKGLEWVS	RISPSGGQTNYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0046-F04	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	YYSMT	WVRQAPGKGLEWVS	SISPSGGGTGYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0037-F03	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	YYSMV	WVRQAPGKGLEWVS	WISSSGGSTNYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807A-M0039-C10	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	YYSMV	WVRQAPGKGLEWVS	WISSSGGSTNYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFREWDY	WGQGTLVTVSS

Table 11: Amino acid sequences of the VL chains of the antibodies identified using the screening strategies of Examples 21 and 22

Antibody Name	VL-FR 1	VL-CDR 1	VL-FR 2	VL-CDR 2	Vl-FR 3	VL-CDR 3	VL-FR 4
807B-M0001-A09	QSVLTQPPSASGTPGQRVTISC	SGSSSNIGTYPVN	WYQQLPGAAPKLLIY	STDQRPS	GVPDRFSGSKSGTSASLAISGLQSEDESDYYC	AAWDDSLNGLWV	FGGGTKVTVL
807B-M0001-B07	QYELTQPPSVSGTPGQRVTISC	SGSSSNIGSEYVY	WFQQLPGTAPRLLIY	RNDQRPS	GVPDRFSGSKSGTSASLAISGLQSEDEADYYC	AAWDDSLPGWC	SGGGTKLTVL
807B-M0001-C10	QYELTQPPSASGTPGQTVTISC	SGSSSNIGTNFVY	WYQQLPGTAPKLLIY	RSIKRPS	GVPDRFSGSKSGTSASLAISGLRSEDEADYYC	AAWDDSLSGVV	FGGGTKLTVL
807B-M0001-G03	QSALTQPPSASGTPGQRVTFSC	SGSSSNIGINSVN	WYQQLPGTAPKLLIY	SNNQRPS	GVPDRFSGSKSGTSASLAISGLRSEDEADYYC	AAWDDSLAGWV	FGGGTKVTVL
807B-M0004-A03	QSELTQPPSASGTPGQRVTISC	SGSSSNIGSNTVN	WYQQLPGTAPKLLIY	NNNQRPS	GVPDRFSGSKSGTSASLAISGLQSEDEADYYC	AAWHDGLNGPV	FGGGTKLTVL
807B-M0004-A05	QDIQMTQSPATLSLSPGERATLSC	KASQSVRAFIA	WYQQKPGQAPRLLIS	GASNRAT	GIPDRFSGGGSGTDFTLTISRLEPEDFAVYYC	QQYGSSRYT	FGQGTKLEIK
807B-M0004-B10	QDIQMTQSPSSLSASVGDRVTITC	RASQSISTYLN	WYQQKPGKAPKLLIF	ATSRLQS	EVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSPPRT	FGQGTKLDIN
807B-M0004-C01	QDIQMTQSPLSLPVSLGQKASISC	RSSQSLVHTDGTTYLS	WFQQRPGQSPRRLVY	KVSDRGS	GVPDRFSGSGSGIDFTLKISRVEAEDVGLYYC	MQGTHWPPT	FGQGTKLEIK
807B-M0004-C04	QDIQMTQSPSSLPVTPGEPASISC	RSSQSLLHSSGYNYLD	WYLQKPGQSPQLLIY	LGSNRAS	GVPDRFTGSGSGTDFTLKISRVEAEDVGVYYC	MQALQTPT	FGGGTKVDIK
807B-M0004-C05	QDIQMTQSPATLSVSPGERATLSC	RASQSVSSNLA	WYQQKPGQAPRLLIY	GASTRAT	GVPARFSGSGSGTDFTLSISSLQPEDFATYYC	QQYAGHPIT	FGQGTRLEIK
807B-M0004-D10	QSELTQPSSASETPGQRVTISC	SGSSSNIGSNLVY	WYQQVPGTALKLLIY	RNDQRPS	GVPDRFSGSKSGTSASLAISGLRSEDEADYFC	VSWDGSLSGWV	FGGGTRLTVL
807B-M0004-F06	QSELTQAASVSGSPGQSITLSC	TGATRDVS	WYQQHPGKAPKLVL	YEVSSRPS	GVSDRFSGSMSGNTASLTISGLQAEDEADYYC	SSTTSRAPRVV	FGGGTKLTVL
807B-M0004-F07	QDIQMTQSPATLSLSPGERATLSC	RASQSVSSYLA	WYQQKPGQAPRLLIY	DAFNRAT	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC	QQRSNWPLT	FGGGTKVEIK
807B-M0004-F10	QDIQMTQSPLSLPVTPGEPASISC	RSSQSLMHRNGHHFFD	WYLQKPGQSPQLLIY	WASNRAP	GVPDRFSGSGSGTDFTLKISRVEAEDVGIYYC	MQALQTPYT	FGQGTKLEIK
807B-M0004-G08	QDIVMTQTPPSLPVNPGEPASISC	RSSQSLVHSDGNTYLN	WFQQRPGQSPRRLIS	KVSNRDS	GVPDRFSGSGAGTDFTLNISRVEAEDVGDYYC	MQVTEFPLT	FGGGTKVEIK
807B-M0004-H03	QDIQMTQSPSSLSASIGDRVTISC	QASQNIDNYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTPRT	FGQGTKVEIK
807B-M0008-A03	QSALTQPASVSGSPGQSITISC	TGTSNDVGGYNYVS	WYQQHPGIAPKVVIY	EVSNRPS	GVSDRFSGSKAGNTASLTISGLQAEDEADYYC	NSYTSSRTWV	FGGGTKVTVL
807B-M0008-A08	QDIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTPLT	FGGGTKVEIK
807B-M0008-B04	QYELTQPPSASGTPGQRVTISC	SGTLSNIGTNIVS	WFQQLPGTAPKLLIY	NDHRRPS	GVPDRFSGSKSATSASLAISGLQSEDEADYYC	AAWDDSLNGVV	FGGGTKLTVL

807B-M0008-B08	QDIQMTQSPSSLSASVGDRVTITC	RASQSISTYLN	WYQEKPGKAPELLIF	AASSLQG	GVPSRFSGSGSGTDFTLTISSLQPEDLATYYC	QQSYDIPLS	FGGGTKVEIK
807B-M0008-D02	QDIQMTQSPATLSLSLGERANFSC	RASEYISTYLA	WYQQKPGQAPRLLIY	DASVRAP	GTPARFSGTGSGTDFTLTISSLQPDDFAVYFC	QERYDWPLT	FGPGTRLDVK
807B-M0008-D05	QSALTQPPSASGTPGQRVTISC	SGSSSNIGRNFVY	WYRQLPGTAPKLLIY	ENNQRPS	GVPDRFSGSKSGTSASLAISGLRSEDEADYHC	AAWDDSLSGLV	FGGGTKLTVL
807B-M0008-E01	QDIQMTQSPGTLSLSPGERGTLSC	RASQSVSSRYLA	WYQQKPGQAPRLLIY	GASSRAP	GIPDRFSGSGSGTDFTLTISSLEPEESAVYYC	QQYGSSPVT	FGGGTKVEIK
807B-M0008-E06	QYELTQPPSASGTPGQRVTISC	SGSSSNIGSNYVY	WHQQLPGTAPKLLIS	RNNQRPS	GVPDRFSGSKSGTSASLAISGLRSEDEADYYC	AAWDDSLSGYV	FGAGTKVTVL
807B-M0008-G11	QSVLTQPASVSGSPGQSITISC	TGASSDVGGSNFVS	WYQQHPGKAPKLIIY	DVSNRPS	GVSNRFSGSKSGNTASLTISGLQAEDDDDTDYYC	SSYTSSSLVV	FGGGTKLTVL
807B-M0009-A06	QSVLTQPPSASGTPGQRVSISC	SGSSSNIGSYYVY	WYQHLPGTAPKLLIY	RNNQRPS	GVPDRFSGSKSGTSASLAISGLRSEDESDYYC	AAWDDRLSTWV	FGGGTKLTVL
807B-M0009-A09	QYELTQPPSVSVSPGQTASITC	SGDKLGDKYAS	WYQQKPDQSPVLVIY	QDRKRPS	GIPERFSGSNSGNTATLTISGTQAMDEADYYC	QAWDSNTVV	FGGGTKLTVL
807B-M0009-B07	QDIQMTQSPGTLSLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQYGSSGWT	FGQGTKVEIK
807B-M0009-C02	QYELTQPPSVSVSPGQTASITC	SGDKLGDKYTS	WHQQKPGQSPVLVIY	QDRKRPS	GIPERFSGSNSGNTATLTISGTQAMDEADYYC	QAWDSNTVV	FGGGTKLTVL
807B-M0009-C03	QSELTQPPSASGTPGQRVTISC	SGSSSNIGSNYVY	WYQQLPGTAPKLLIY	RNNQRPS	GVPDRFSGSKSGTSASLAISGLQSEDEADYYC	AAWDDSLNAWV	FGGGTKLTVL
807B-M0009-F06	QDIQMTQSPLSLPVTPGEPASISC	KSSQSLLHSNGYNYLD	WYLQKPGQSPQLLIS	LGSNRAS	GVPARFSGSGSGTDFTLKISRVEAEDVGVYYC	MQALQTIT	FGQGTRLEIK
807B-M0009-G03	QSVLTQSPSASASLGASVRVTC	TLSSGHSNYDIA	WHQQQPEKGPRY	LMKLNSD	GIPDRFSGSSSGTERYLTISSLQSEDEADYYC	QTWGTGLRV	FGGGTKLTVL
807B-M0023-C03	QSELTQPPSVSVSPGQTATITC	SGYDLGAKFVS	WYQQKSGQSPVLVM	YQDNKRPS	GIPERFSGSNSGNTATLTISGTQAMDEADYYC	QVWDSPSYI	FGTGTTVTVL
807B-M0023-G05	QDIQMTQSPGTLSLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDRFSGSGSGTDFTLTISSLHPEDFATYFC	QQGNSFPIT	FGQGTRLEIK
807B-M0024-H08	QDIQMTQSPSSLSASVGDRVSITC	RASQSISSHLN	WYQQKPGKVPKVLIY	GASRLQS	GVPSRFSGSGSGTDFTLTINSLQPEDFATYYC	QQSYRAPVFT	FGPGTKVDVK
807B-M0025-B05	QSVLTQPPSASGTPGQRVTISC	SGSSSNIGRNPVN	WYQHLPGTAPKLLIY	GDNQRPS	GVPDRFSGSRSGTSASLAISGLQSEDEADYYC	AAWDDSLYGPV	FGGGTKLTVL
807B-M0027-E08	QSALTQPPSVSVSPGQTASITC	AGDELGNKYAS	WYQQKPGQSPVLVIY	QDRKRPS	GIPERFSGSHSGNTATLTISGTQALDEADYYC	QSWDSSSVI	FGGGTKVTVL
807B-M0042-A05	QDIQMTQSPSAMSASVGDRVTITC	RASQGISNYLA	WFQQKPGKVPKRLIY	AASSLQS	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	LQHNSYPLT	FGGGTKVEIK
807B-M0042-B05	QSALTQPPSASGTPGQRVTISC	SGSSSNIGSHYVY	WYQQLPGTDPKLLIY	KSIQRPS	GVPDRFSGSKSGTSASLAISGLRSDDEGDYYC	AAWDDSLSGSYV	FGTGTKVTVL
807B-M0046-E03	QDIQMTQSPSSVSASVGDRVTLTC	RASQDISSWLA	WYQQKPGKAPKLLIY	AASRLQS	GVPSRFSGSGSGTDFSLTISSLQPDDFATYYC	QQSHSFPLS	FGGGTKVEIK

807B-M0050-A04	QDIQMTQSPSSLSVSPGERATLSC	RASQTISNDLA	WYQQTPGQAPRLLIY	GASSRAT	GIPDRFSGSGSGTDFTLTISNLQPEDFATYFC	QQADSFPLT	FGGGTKVEIK
807B-M0050-B09	QDIQMTQSPDSLAVSLGERATINC	QSSQSVLYSSNNKNYLA	WYQQKPGQPPKLLIY	GASTRES	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	QQYYTTPLT	FGGGTKVEIK
807B-M0050-E04	QDIQMTQSPSSVSASVGDRVTITC	RASQGISSWLA	WYQQKPGKAPKLLIY	PASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGTSFPLT	FGGGTKVEIK
807B-M0050-G01	QDIQMTQSPSTLSASVGDRVTITC	RASQSISTWLA	WYQQKPGKAPKLLIY	KAFSLEG	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	QQSYSPPLT	FGGGTKVDIR
807B-M0050-G05	QDIQMTQSPSSLSASVGDRVTITC	RASQGISNYLA	WYQQKPGKVPKLLIY	AASTLQS	GVPSRFSGSGSGTDFTLTISSLQPEDVATYYC	QNYNRAPRT	FGQGTKVEIK
807B-M0050-H05	QDIQMTQSPPSVSASVGDRVTITC	RASQVITRWLA	WYQQKPGQAPKLLIY	SASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQATSFPLT	FGGGTKVEIK
807B-M0050-H10	QDIQMTQSPLSLSASVGDRVTITC	RASQSISSYLN	WYQHKPGKAPRLLIY	GASSLQN	GVPSRFTGSGTGTDFTLTISSLQPEDFATYYC	QQSFTTPLT	FGGGTKVEIK

Table 12: Amino acid sequences of the VH chains of the antibodies identified using the screening strategies of Examples 21 and 22

Isolate Name	VH-FR 1	VH-CDR 1	VH-FR 2	VH-CDR 2	VH-FR 3	VH-CDR 3	VH-FR 4
807B-M0001-A09	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	RYPMF	WVRQAPGKGLEWVS	SISSSGGYTVYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	VGKGAYYYAMDV	WGQGTTVTVSS
807B-M0001-B07	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	FYGMV	WVRQAPGKGLEWVS	SISPSGGYTLYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	DGRRPHYGSGRWAY	WGQGTLVTVSS
807B-M0001-C10	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	QYVMF	WVRQAPGKGLEWVS	SISSSGGKTSYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	RLKIYDSSGYYYYYGMDV	WGQGTTVTVSS
807B-M0001-G03	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	SYAMS	WVRQAPGKGLEWVS	SISPSGGFTPYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	VGKGAYYYAMDV	WGQGTTVTVSS
807B-M0004-A03	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	RYLMM	WVRQAPGKGLEWVS	VISPSGGRTWYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCVR	SIAAAGTDY	WGQGTLVTVSS
807B-M0004-A05	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	NYFMI	WVRQAPGKGLEWVS	WISPSGGTTQYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	EAGY	WGQGTLVTVSS
807B-M0004-B10	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	KYVMI	WVRQAPGKGLEWVS	SISPSGGDTTYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLGSNWGTGVWN	WGQGTLVTVSS
807B-M0004-C01	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	NYNMH	WVRQAPGKGLEWVS	SIYSSGGTTLYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	LVADEWIDAFDI	WGQGTMVTVSS
807B-M0004-C04	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	AYYMG	WVRQAPGKGLEWVS	VIRPSGGKTKYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GPHGQGGVDS	WGQGTLVTVSS
807B-M0004-C05	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	EYFMT	WVRQAPGKGLEWVS	SIRPSGGKTRYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	VSRYYNNGAYRLDAFDI	WGPGTVVTVSS
807B-M0004-D10	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYQMS	WVRQAPGKGLEWVS	VISPSGGRTIYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	QGLLTAFDI	WGQGTMVTVSS
807B-M0004-F06	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	AYRMA	WVRQAPGKGLEWVS	YISSSGGVTSYADSVKG	RFTISRDNSKNTLYLQMKSLRAEDTAVYYCAR	GTHLPGVDY	WGQGTLVTVSS
807B-M0004-F07	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	EYYMT	WVRQAPGKGLEWVS	SIRPSGGATRYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807B-M0004-F10	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	GYIMA	WVRQAPGKGLEWVS	GIGSSGGLTAYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	EAGY	WGQGTLVTVSS
807B-M0004-G08	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	TYAMA	WVRQAPGKGLEWVS	SIRSSGGVTKYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GGAVAGY	WGQGTLVTVSS
807B-M0004-H03	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	SYPMV	WVRQAPGKGLEWVS	GIWSSGGLTYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	EGSAGVVKGPARYYYYMDV	WGKGTTVTVSS
807B-M0008-A03	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	HYTMY	WVRQAPGKGLEWVS	GISPSGGVTPYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	AGSGGSFDY	WGQGTLVTVSS
807B-M0008-A08	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	KYLMM	WVRQAPGKGLEWVS	YIWPSGGTDYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	VRTSRINGSGFDY	WGQGTLVTVSS
807B-M0008-B04	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	RYPMS	WVRQAPGKGLEWVS	SISPSGGPTSYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	SGPYYFDY	WGQGTLVTVSS
807B-M0008-B08	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	HYPMS	WVRQAPGKGLEWVS	SISPSGGFTMYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	FEYSSSSGISWFDP	WGQGTLVTVSS

807B-M0008-D02	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	KYGMT	WVRQAPGKGLEWVS	SIRPSGGITKYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	ENYGPDY	WGQGTLVTVSS
807B-M0008-D05	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	TYHMF	WVRQAPGKGLEWVS	GISSSGGSTRYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	VSVTTNAFDI	WGQGTMVTVSS
807B-M0008-E01	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	TYAMT	WVRQAPGKGLEWVS	SISSSGGGTKYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	HGYSSGWPPFDY	WGQGTLVTVSS
807B-M0008-E06	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	HYPMS	WVRQAPGKGLEWVS	SIVPSGGYTLYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	HNRAIGTFDY	WGQGTLVTVSS
807B-M0008-G11	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	SYAMI	WVRQAPGKGLEWVS	GISPSGGQTVYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	AGSGGSFDY	WGQGTLVTVSS
807B-M0009-A06	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	VYNMV	WVRQAPGKGLEWVS	VISPSGGSTYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DRGYCSGNTCYIDAFDI	WGQGTMVTVSS
807B-M0009-A09	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	RYPMA	WVRQAPGKGLEWVS	GISSSGGLTSYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	FVGAKPADY	WGQGTLVTVSS
807B-M0009-B07	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	FYWMV	WVRQAPGKGLEWVS	GISPSGGPTKYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807B-M0009-C02	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	SYPMT	WVRQAPGKGLEWVS	GISSSGGSTAYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	VTGGDFDY	WGQGTLVTVSS
807B-M0009-C03	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	KYQMT	WVRQAPGKGLEWVS	VISSSGGDTAYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DRGYCSGNTCYIDAFDI	WGQGTMVTVSS
807B-M0009-F06	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	PYWMF	WVRQAPGKGLEWVS	GIVSSGGMTGYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	VGMSTYAFDI	WGQGTMVTVSS
807B-M0009-G03	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	PYRMA	WVRQAPGKGLEWVS	SISPSGGHTGYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	ESDGTTSAYFDY	WGQGTLVTVSS
807B-M0023-C03	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	SYMMG	WVRQAPGKGLEWVS	YIYPSGGWTYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GRSWGRYFQH	WGQGTLVTVSS
807B-M0023-G05	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	MYWMG	WVRQAPGKGLEWVS	SISPSGGFTMYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GLYR	WGQGTLVTVSS
807B-M0024-H08	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	AYNMD	WVRQAPGKGLEWVS	SIYPSGGHTNYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GKRIAARGGYYFDY	WGQGTLVTVSS
807B-M0025-B05	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	NYSMV	WVRQAPGKGLEWVS	SIVPSGGFTLYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	HGSSWTFDY	WGQGTLVTVSS
807B-M0027-E08	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	NYRME	WVRQAPGKGLEWVS	SIWSSGGLTKqADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GLYR	WGQGTLVTVSS
807B-M0042-A05	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	HYQMK	WVRQAPGKGLEWVS	SIGSSGGSTSYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GPL	WGQGTLVTVSS
807B-M0042-B05	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	NYHMD	WVRQAPGKGLEWVS	SISPSGGITKYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTSVYYCAG	GVGATAGI	WGQGTMVTVSS
807B-M0046-E03	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	PYAMI	WVRQAPGKGLEWVS	YISPSGGKTQYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	DQQTDYFDY	WGQGTLVTVSS
807B-M0050-A04	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	QYNMN	WVRQAPGKGLEWVS	GISSSGGPTVYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	FRCTSTSCFSDY	WGQGTLVTVSS
807B-M0050-B09	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	NYGML	WVRQAPGKGLEWVS	VISSSGGYTFYADSVKG	RFTISRDNSKNSLYLQMNSLRAEDTAVYYCAR	VGATGPFDI	WGQGTMVTVSS

807B-M0050-E04	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	RYSMM	WVRQAPGKGLEWVS	GISPSGGPTSYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	ENIEYSSSFPNMGRPHYYYYYGMDV	WGQGTTVTVSS
807B-M0050-G01	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	RYEMY	WVRQAPGKGLEWVS	VISSSGGTTFYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GGDNWNYLSV	WGQGTTVTVSS
807B-M0050-G05	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	PYPMD	WVRQAPGKGLEWVS	VISSSGGTTYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	VGMSTYAFDI	WGQGTMVTVSS
807B-M0050-H05	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	NYVMH	WVRQAPGKGLEWVS	SIGPSGGGTEYADSVKG	RFTISRDNTKNTLYLQMNSLRAEDTAVYYCAR	DRGYCSGNTCYIDAFDI	WGQGTMVTVSS
807B-M0050-H10	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	TYPMQ	WVRQAPGKGLEWVS	VISSSGGYTQYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAS	MRVDYGDNYGMDV	WGQGTTVTVSS

Table 13

Isolate Name	Peptide	Enrichment		Peptide Mapping										bCTD	Fab IHC	Group
		VH	CDR3	1	2	3	4	5	6	7	8	9	10			
807B-MO001-A09	E22-P1	6	8	+												
807B-MO001-B07	E22-P1	2	2	+					+					+	+/-	4
807B-MO001-C10	E22-P1	1	1	+												
807B-MO001-G03	E22-P1	2	8	+												
807B-MO042-A05	E22-P2	1	1		+											
807B-MO042-B05	E22-P2	7	7		+											
807B-MO050-A04	E22-P3	19	19			+										
807B-MO050-B07	E22-P3	20	20			+								+		
807B-MO050-B09	E22-P3	4	4			+										
807B-MO050-E04	E22-P3	1	1			+										
807B-MO050-G01	E22-P3	2	2			+										
807B-MO050-G05	E22-P3	2	3			+										
807B-MO050-H05	E22-P3	1	8			+										
807B-MO050-H10	E22-P3	1	1			+										
807B-MO004-A03	E22-P4	5	5				+					+		+	+	5
807B-MO004-A05	E22-P4	2	3				+								+/-	1
807B-MO004-B10	E22-P4	3	3				+									
807B-MO004-C01	E22-P4	2	2				+									
807B-MO004-C04	E22-P4	1	1				+								+/-	1
807B-MO004-C05	E22-P4	1	1				+								+/-	1
807B-MO004-D10	E22-P4	1	1				+									
807B-MO004-F06	E22-P4	2	2				+								+/-	1
807B-MO004-F07	E22-P4	1	4				+									
807B-MO004-F10	E22-P4	1	3				+					+			+/-	2
807B-MO004-G08	E22-P4	1	1				+									
807B-MO004-H03	E22-P4	1	1				+					+			+	2
807B-MO023-C03	E23-P4	1	1				+									
807B-MO023-G05	E23-P4	1	149				+									
807B-MO024-H08	E23-P4	4	4				+									
807B-MO046-E03	E22-P7	3	3							+						
807B-MO008-A03	E22-P8	2	3								+					
807B-MO008-A08	E22-P8	1	1								+					
807B-MO008-B04	E22-P8	2	2								+					
807B-MO008-B08	E22-P8	1	1								+			+		
807B-MO008-D02	E22-P8	5	5								+					
807B-MO008-D05	E22-P8	2	2								+					
807B-MO008-E01	E22-P8	2	2								+					
807B-MO008-E06	E22-P8	1	1								+					
807B-MO008-G11	E22-P8	1	3								+					
807B-MO008-G12	E22-P8	1	1								+					
807B-MO025-B05	E23-P8	1	1								+			+		
807B-MO027-E08	E23-P8	148	149				+				+			+	+	6
807B-MO009-A06	E22-P9	5	8				+					+				
807B-MO009-A09	E22-P9	15	15									+				
807B-MO009-B07	E22-P9	4	5	+	+	+/-	+	+	+	+	+	+	+	+		
807B-MO009-C02	E22-P9	13	13									+				
807B-MO009-C03	E22-P9	2	8				+					+		+	+/-	5
807B-MO009-F06	E22-P9	1	3									+			+/-	3
807B-MO009-G03	E22-P9	1	1				+					+				

Table 14: Amino acid sequences of the VL chains of the antibodies identified using the screening strategy of Example 23

Isolate Name	FR 1	CDR 1	FR 2	CDR 2	FR 3	CDR 3	FR 4
807B-M0011-C07	QYELTQPPSASGSPGQSVTISC	TGTSSDVGTYKYVS	WYQQHPDKAPRLIIY	EVNRRPS	GVPDRFSGSKSGNTASLTISGLQAEDEADYYC	YSHATGNNYV	FGTGTKVTVL
807B-M0012-C09	QSELTQPPSASGTPGQRVTISC	SGTLSNIGTNIVS	WFQQLPGTAPKLLIY	NDHRRPS	GVPDRFSGSKSATSASLAISGLQSEDEADYYC	AAWDDSLNGVV	FGGGTKLTVL
807B-M0012-D09	QDIQMTQSPATLSLSPGERATLSC	RASQSVSSYLA	WYQQKPGQAPRLLIY	DASNRAT	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC	QQRSNWPRYT	FGQGTKLEIK
807B-M0012-F10	QDIQMTQSPSFLSASVGDRVTITC	RASQGISNYLA	WYQQKPGKAPKLLIY	VASALQS	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	QQYYSYSA	FGQGTRVEIK
807B-M0012-F12	QDIQMTQSPATLSVSPGERATLSC	RASQSVSSNLA	WYQQKPGQAPRLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTVSSLQPEDFATYYC	QQTYSTPWT	FGQGTKLEIK
807B-M0012-G05	QDIQMTQSPGTLSLSPGERATLSC	RASRSLFSTYLA	WYQQKPGQPPRLLIY	GASTRAT	GIPDRFSGSGSGTDFTLTISRLEPEDSALYYC	QQYVSSQLT	FGGGTKVEIK
807B-M0013-A12	QDIQMTQSPSTLSASVGDRVTITC	RASQSISRWLA	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTPLT	FGGGTKVEIK
807B-M0013-B04	QDIQMTQSPSSLPASVGDRVTITC	RASQNIVTYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSMSSWT	FGQGTNLEIK
807B-M0013-C03	QDIQMTQSPDSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTPPYT	FGQGTKLEIK
807B-M0013-F06	QYELTQPPSASGTPGQRVTISC	SGSGSNIGSNLVY	WYQQLPGTAPKLLIY	RNTQRPS	GVPDRFSASKSGTSASLAISGLRSEDEADYHC	ATWDDSLGGVV	FGGGTKLTVL
807B-M0013-G05	QDIQMTQSPGTLSLSPGERATLSC	RASQSLSSSYLA	WYQQKPGQPPRLLIY	GASRRAT	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QHYGRSPLFT	FGPGTTVDIK
807B-M0014-D07	QDIQMTQSPATLSLSPGESTTLSC	RASQSVSRYVA	WYQQKPGQSPRLVIY	DASNRAT	GIPARFSGSGSGTDFTLTITSLEPEDFGIYYC	LQRSNWPFT	FGPGTKVEIK
807B-M0014-D09	QDIQMTQSPSSFSASTGDRVTITC	RASQGVGSYLA	WYQQKPGKAPKLLIY	GAYTLQS	GVPSRFSGSGSGTDFTLIISGLQSEDFATYYC	QQYYSYPFT	FGPGTKVDIK
807B-M0014-E08	QDIQMTQSPSSLSASVGDRVTITC	RASQDIRDDLG	WYQQKPGKAPKRLIY	AASSLQS	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	QQHNNYPSFT	FGPGTRLDIK
807B-M0014-F07	QDIQMTQSPSSLSASVGDRVAITC	RASQSIDTYLN	WYQQKPGKAPKLLIY	AASKLED	GVPSRFSGSGTGTDFTLTIRSLQPEDFASYFC	QQSYSSPGIT	FGPGTKVEIK
807B-M0016-C06	QDIQMTQSPSSLSASVGDRVTITC	RASQSINTYLN	WYQQKPGQAPKLLIY	ASSTLQR	GVPSRFSGSGSGTDFTLTISSLQLEDFATYFC	QQSYSPPLYT	FGQGTKLDLK
807B-M0016-D01	QDIQMTQSPSSLSASVGDRVTITC	RASQSIDNYLN	WYQQKPGIAPKLLIY	TASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYTTPHT	FGQGTKLEIR
807B-M0016-D08	QDIQMTQSPSSLSASVGDRVAITC	RASQSISSFLN	WYQQKPGKAPNLLIY	GASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTPYT	FGQGTKLEIK
807B-M0016-E01	QDIQMTQSPSSLSASVGDRITITC	RASQSIGRYLN	WYQQKPGKAPKLLIY	TASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSFTTPHT	FGLGTKLEIE

807B-M0016-F04	QDIQMTQSPATLSA SVGDRVTFTC	RASQSVNNWVA	WYQQKPGGAPEGLIY	KASHLQS	GVPSRFSGGGSGTVFTLTI TSLQPDDFATYYC	QQYQTYPYT	FGQGTRLDMK
807B-M0016-F05	QDIQMTQSPSSLSA SVGDSVTITC	RASQSISTYLN	WYQQKPGKAPKLLIS	APSRLQS	GVPSRFSDSGSGTDFTLAI SSLQPEDFATYYC	QQSYSTPVT	FGQGTKLEIK
807B-M0016-F08	QSALTQPRSVSGSP GQSVTISC	TGTSSDVGGYNYVS	WYQQHPGKAPKLMIY	DVSKRPS	GVPDRFSGSKSGNTASLTI SGLQAEDEADYYC	CSYAGNYSVV	FGGGAKLSVL
807B-M0017-B05	QDIQMTQSPGTLSL SPGERATLSC	RASQSVHSSYLA	WYQQKPGQAPRLLIY	GTSSRAT	GIPDRFSGNGFGTDFTLTI SRLEPEDFAVYYC	QQYGSSPIT	FGQGTRLEIK
807B-M0017-B06	QYELTQPPSASGSP GQSVTISC	TGTSSDVGAYNYVS	WYKIHPGKAPKLMIY	DVNNRPS	GVSNRFSGSKSGNTASLTI SGLQAEDEADYYC	CSYAGSSTQV	FGTGTKVTVL
807B-M0017-E05	QDIQMTQSPGTLSL SPGERATLSC	RASQSVTSSYLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDRFSGSGSGADFTLTI SRLEPEDFAVYYC	QQYGTSPYT	FGQGTKLEIK
807B-M0018-C12	QSELTQPPSASGSP GQTVTISC	TGSSRDIGNYNYVS	WYQQFPGKAPKLIIY	DVRKRPS	GVSDRFSGSKSGNTAFLT VSGLQTEDEADYFC	GSYTGTNNV	FGPGTSVTVL
807B-M0018-E09	QDIQMTQSPGTLSL SPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDRFSGSGSGTDFTLTI SRLEPEDFAVYYC	QQYGSSRVT	FGGGTKVEIK
807B-M0018-G02	QDIQMTQSPSSLSA SIGDRVTITC	RASQDIRDDLG	WYQQKPGKAPKRLIY	AASSLQS	GVPSRFSGSGSGTEFTLTI SSLQPEDFATYYC	LQHNTFPSFT	FGPGTKVDIK
807B-M0019-A04	QDIQMTQSPGTLSL SPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDRFSGSGSGTDFTLTI SRLEPEDFAVYYC	QQYGSSSIT	FGQGTRLEIK
807B-M0019-A10	QDIQMTQSPLSLSAS IGDRVTITC	RASQDIRSDLG	WYQQKPGKAPKLLIY	GASTLQS	GVPSRFSGSGSGADFTLII SNLQPEDFATYYC	LQDYNYPRT	FGQGTKVEIK
807B-M0019-C01	QSALTQPASVSGSP GQPITISC	SGTSSDVGGYNYVS	WYQQHPGKAPKLVIY	DVSNRPS	GISYRFSGSKSVNTASLTIS GLQAEDEADYFC	SSYTSNSTLV	FGGGTQADRP
807B-M0019-F06	QSELTQPASVSGSP GQSITISC	TGTSSDVGSYNLVS	WYQQHPGKAPKLMIY	EGSKRPS	GVSNRFSGSKSGNTASLTI SGLQAEDEADYYC	CSYAGSSTLV	FGGGTKLTVL
807B-M0019-G07	QDIQMTQSPATLSA SVGDRVTITC	RASQGLASWLA	WYQQKPGKAPNLLIY	KASNLKS	GVPSRFSGSESGTEFTLTI SSLQPDDFATYFC	HQYYSNSWT	FGQGTKVEIK
807B-M0020-D01	QDIQMTQSPSSLSA SVGDRVSIT.	RASQTIRDYLH	WYQQKPGKAPKLLIY	AASSLQV	GVPSRFSGSGSGTDFTLTI SSLQPEDLATYYC	QQTYSTLIT	FGQGTRLEIK
807B-M0020-F06	QDIQMTQSPGTLSL SPGERATLSC	RASQSISYNYLA	WYQQKPGQAPRLLIY	DASNRAT	GIPARFSGSGSGTDFTLTI SSLEPEDFAVYYC	QQRSNWPPGLT	FGGGTKVEIK
807B-M0020-F12	QDIQMTQSPSSLSA SVGDRVTITC	RASQDIRNYLA	WFQQKPGKAPKSLIY	GASSLQG	GVPSKFSGSGSGTDFTLTI SGLQPEDFATYYC	QQYNSYPLT	FGGGTKVEVK
807B-M0020-G01	QSELTQPASVSGSP GQSITISC	TGTSSDVGSYNLVS	WYQQHPGKAPKLMIY	EGSKRPS	GVSNRFSGSKSGNTASLTI SGLQAEDEADYYC	CSYAGSSTYV	FGTGTKVTVL
807B-M0079-B09	QDIQMTQSPSSLSA SVGDRVTITC	RASQSISSYLN	WYQQKPGNAPRLLIY	SASTLNS	GVPSRFSGSGSGTHFTLTI SSLQPEDFGIYYC	QQANSLPFT	FGQGTKLEIK
807B-M0079-D10	QSALTQPPSVSVSP GQTASITC	AGDELGNKYAS	WYQQKPGQSPVLVIY	QDRKRPS	GIPERFSGSHSGNTATLTI SGTQALDEADYYC	QSWDSSSVI	FGGGTKVTVL
807B-M0079-H01	QDIQMTQSPSSLSA SVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTI SSLQPEDFATYYC	QQSYSTPFT	FGPGTKVDIK
807B-M0079-H05	QDIQMTQSPSSLSA SVGDRVTITC	RASQGINSWLA	WYQQRPGKAPRSLIY	AATNLQN	GVPSRFSGSGSGTLFTLTI NNLQPEDFATYYC	QQYQNYPYT	FGQGTKLDIE

807B-M0080-A02	QDIQMTQSPLSLSASVGDRVTIPC	RASQSISTYLN	WYQQKPGKAPKILIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYFC	QQSYTTPLT	FGGGTKVEIK
807B-M0080-C04	QSALTQPPSASGTPGQTVAISC	SGSTSNIGSNNVN	WYQQLPGTAPKLLMY	TTNYRPS	GVPARFSGSKSGTSASLAISGLQSEDEADYYC	AAWDDSLNGPNVV	FGGGTKLTVL
807B-M0080-F10	QDIQMTqSPSSVSASIGDRVTITC	RASQGISIWLA	WYQQKPGKAPKLLIY	GASSLQS	GAPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQANSFPLT	FGGGTKVEIK
807B-M0081-C03	QDIQMTQSPSTLSASVGDRVTITC	RASQSINRWLA	WYQQKPGKAPKLLIY	KASNLES	GAPSRFSGSGSGTEFTLTISSLQPDDFGTYYC	QQYHSYPWT	FGQGTKVDVK
807B-M0081-C05	QSALTQPPSVSGAPGQRVTISC	TGSSSNIGAPYDVH	WYQQVPGTAPKVLIY	GNNHRPS	GVPDRFSGSKSGTSASLAISGLQAEDEAHYYC	QSYDSSLSGPI	FGGGTTLTVL
807B-M0081-D08	QDIQMTQSPSSVSASVGDRVTITC	RASQGISSWLA	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQANSFPPT	FGQGTKVEIQ
807B-M0081-E08	QDIQMTQSPSSLPASVGDRVTITC	RASRNIGKYLN	WYQQIRGRAPRLLVY	LASSVQT	GVPPRFSGSGSGTDFSLIISSLQPEDFATYYC	QQSYAAPLT	FGGGTKVEIK
807B-M0081-F12	QDIQMTqSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTEFSLSISSLQPEDFATYYC	QQANSFPLT	FGGGTKVEIK
807B-M0081-G04	QDIQMTQSPDTLSLSPGERATLSC	RASQSISTSLA	WYHQRPGQAPRLLIY	DASNRAT	GVPARFSGTGSGTDFTLTISSLEPEDFAVYYC	QQRSNWPYT	FGQGTKLEIK
807B-M0081-G11	QDIQMTQSPSSLSASVGDRVAITC	RASQSIDTYLN	WYQQKPGKAPKLLIY	AASKLED	GVPSRFSGSGTGTDFTLTIRSLQPEDFASYFC	QQSYSSPGIT	FGPGTKVEIK
807B-M0081-H03	QYELTQPPSASGSPGQSVTISC	TGTSSDVGGYNYVS	WYQQHPGKAPKFMIY	EVNKRPS	GVPDRFSGSKSGNTASLTVSGLQAEDEADYYC	SSYAGRNFVV	FGGGTKLTVL
807B-M0081-H07	QDIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLIISDLQPEDFATYYC	QQSYTTPFT	FGPGTTVDIK
807B-M0082-B07	QSALTQPASVSGSPGQSITISC	TGTSSDVGGYNYVS	WYQQHPGKAPKLMIY	DVSNRPS	GVSNRFSGSKSGNTASLTISGLQAEDEADYYC	SSYTSRSTYV	FGTGTKVTVL
807B-M0082-E01	QDIQMTQSPSSLSASVGDRVAITC	RASQSIDTYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTVSSLQPEDFATYYC	LQSNTFPFT	FGPGTKVDIT
807B-M0082-E08	QDIQMTQSPSSLSASIGDRVTITC	RASQSISSYLN	WYQQKQGKAPKLLMF	AASSLKS	GVPSRFSGSGSGTDFTLTISNLQPEDFATYYC	QQTYSSPWT	FGQGTKVEIR
807B-M0082-H06	QDIQMTQSPSSLSASVGDRVTVTC	RASQGIRNNLA	WYQQRPGKAPKRLIY	GASNLHS	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	LQHNNYPYS	FGQGTKLEIK
807B-M0083-B10	QDIQMTQSPSSVSASVGDRVTIIC	RASQDIHTWLA	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTPRT	FGQGTKVEIK
807B-M0083-E10	QDIQMTQSPGTLSLSPGERATLSC	RASQSISSRYLA	WYQQKAGQAPRLLMY	GASRAT	GIPARFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYEYPLT	FGQGTKLEIK
807B-M0083-E11	QSALTQPPSVSVAPGQTARITC	GGNNIGTKIVN	WYQQRPGQAPVVVVY	DNSDRPS	GIPERFSGSNSGNTATLTISRVEAGDEADYYC	QLWDSSSDHPI	FGTGTKVTVL
807B-M0084-C03	QYELTQPPSVSVAPGQTARISC	GGSNIGSKSVH	WYQQKSGQAPVLVVY	DDSDRPS	GIPERFFGSNSGRTATLTISGVEVGDEADYYC	QVWDSSSDDYV	FAAGTKLSVL
807B-M0084-C11	QDIQMTQSPSSLSASVGDRVTITC	RASQSIATFLN	WYQQKPGKAPNLLIS	GAFNLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QHSYGTPT	FGQGTKVEIK
807B-M0084-E07	QDIQMTQSPSSLPASVGDRVTITC	RASQSISRYLN	WYQQKPGKAPKVMIY	DASTLQS	GVPSRFSGSGSGTDFTLTISNLQPEDFATYYC	QQSYITPRT	FGQGTKVEIK

807B-M0084-F03	QDIQMTQSPATLSV SPGERATLSC	RASQSVSNNLA	WYQQKPGQAPRLLIY	AASTRAT	GIPARFSGSGSGTDFTLTI SSLEPEDFAVYYC	QQYYYTPPT	FGRGTKVEIN
807B-M0084-F08	QDIQMTQSPATLSV SPGARATLSC	RASQSVRNNLA	WYQQKPGQAPRLLIY	GASTRAT	DIPARFSGGGSGTEFTLTI SSLQPDDFATYYC	QHEET	FGQGTKVEIK
807B-M0084-H05	QSALTQPPSVSAAP GQRVTISC	SGGTSNIQYNGVN	WYQQLPGKAPKLLIY	FDDLLPS	GVSDRFSGSKSGTSASLAI SGLRSEDEADYYC	AAWDDSLSGVV	FGGGTKLTVL
807B-M0085-B12	QDIQMTQSPSSVSA SVGDRVTITC	RASQGISSWLA	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTI SSLQPEDFATYYC	QQANSFPLT	FGGGTKVEIK
807B-M0085-C01	QDIQMTQSPATLSV SPGERATLSC	RASQSVSSNLA	WYQQKPGQAPRLLIY	GASTRAT	GIPARFSGSGSGTDFTFTI SSLQPEDIATYFC	QQYINLPIT	FGQGTRLEIK
807B-M0085-E10	QDIQMTQSPSSLSA SVGDRVTITC	QASQDISKYLN	WYQQRPGKAPELLIY	DASNLEP	GVPSRFSGSGSGTHFTFTI SSLQPEDFATYYC	QQFDNFPLT	FGPGTRLDIK
807B-M0085-G03	QDIQMTQSPLSLSAS VGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTI SSLQPEDFATYYC	QQSYSTPLYT	FGQGTKLEIK
807B-M0085-G07	QDIQMTQSPGTLSL SPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDRFSGSGSGTDFTLTI SRLEPEDFAVYYC	QSGVT	FGGGTKVEIK
807B-M0085-G08	QDIQMTQSPGTLSL SPGERATLSC	RASQSVSRSSLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDRFSGSGSGTDFTLTI SRLEPEDFAVYYC	QQYGNSPGGT	FGQGTKVEIK
807B-M0086-C06	QSALTQPPSASGTP GQKVTISC	SGGSSNIGSNIVN	WYQQVPGMAPKLLYT NNRRPS	TNNRRPS	GVPDRFSGSKSGTSASLAI SGLRSEDEADYYC	AAWDDSLSGGV	FGGGTKLTVL
807B-M0086-D03	QDIQMTQSPSSLSA SVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	VASSLQS	GVPSRFSGSGSGTDFTLTI SSLQPEDFATYYC	QQSYSIPPT	FGQGTRVEIK
807B-M0086-E08	QDIQMTQSPGTLSL SPGERATLSC	SVSQSVSSNYLA	WYQQKPGQSPRLLIY	GASARAT	GIPDRFSGSGSRTDFTLTI SRLEPEDFAVYYC	QQYVTTPPT	FGQGTKVEIK
807B-M0086-G03	QDIQMTqSPSFLSAS VGDRVSVTC	RASQVLRRPLA	WYQQKAGKAPKLLIS	AFSILES	GVPSRFSAGGSGTEFTLTI NSLQAEDFATYYC	QQVSSYPLT	FGGGPRVEIK

Table 15: Amino acid sequences of the VH chains of the antibodies identified using the screening strategy of Example 23

Isolate Name	FR1	CDR 1	FR 2	CDR 2	FR 3	CDR 3	FR 4
807B-M0011-C07	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYSMD	WVRQAPGKGLEWVS	GIGPSGGRTRY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807B-M0012-C09	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYTMD	WVRQAPGKGLEWVS	GISPSGGATNY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	GLRYFDFYYYYGMD V	WGQGTTVTVSS
807B-M0012-D09	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	LYQMA	WVRQAPGKGLEWVS	SISSSGGLTDY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0012-F10	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYNMS	WVRQAPGKGLEWVS	YIYPSGGITIYA DSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	ERGTIFNDAFDI	WGQGTMVTVSS
807B-M0012-F12	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYDMD	WVRQAPGKGLEWVS	SIYPSGGLTGY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807B-M0012-G05	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYRMT	WVRQAPGKGLEWVS	SISPSGGVTLYA DSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0013-A12	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	HYGMS	WVRQAPGKGLEWVS	SIRSSGGRTWY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAK	GSLSSGWDY	WGQGTLVTVSS
807B-M0013-B04	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	HYDMV	WVRQAPGKGLEWVS	VIVPSGGATAY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	EDFWSGLEDV	WGKGTTVTVSS
807B-M0013-C03	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYNMA	WVRQAPGKGLEWVS	SISPSGGHTKY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0013-F06	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	KYVMT	WVRQAPGKGLEWVS	VISSSGGPTDY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	WGVRGVIPFDY	WGQGTLVTVSS
807B-M0013-G05	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYSMI	WVRQAPGKGLEWVS	YIGPSGGPTRY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807B-M0014-D07	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYTMT	WVRQAPGKGLEWVS	SISSSGGVTKY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	GRWLAPFDY	WGQGTLVTVSS
807B-M0014-D09	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	YYIMA	WVRQAPGKGLEWVS	SISPSGGGTVY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	ATCTGGSCYRFDY	WGQGTLVTVSS
807B-M0014-E08	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYAMD	WVRQAPGKGLEWVS	SIYPSGGWTEY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	GLGMDV	WGQGTTVTVSS
807B-M0014-F07	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYDMF	WVRQAPGKGLEWVS	SISPSGGFTQY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	QEVWQWPAQFDS	WGQGTLVTVSS
807B-M0016-C06	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	LYGMS	WVRQAPGKGLEWVS	SIGPSGGHTFY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0016-D01	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYNMG	WVRQAPGKGLEWVS	GISPSGGTTTY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0016-D08	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYGMV	WVRQAPGKGLEWVS	SIYPSGGTTQY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0016-E01	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYDMQ	WVRQAPGKGLEWVS	SISSSGGLTTYA DSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS

807B-M0016-F04	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	NYNMH	WVRQAPGKGLEWVS	VISPSGGGTWYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DFFTSYFDY	WGQGTLVTVSS
807B-M0016-F05	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYRMT	WVRQAPGKGLEWVS	SISPSGGVTLYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0016-F08	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	MYHMG	WVRQAPGKGLEWVS	GISPSGGTTTYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0017-B05	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYTMF	WVRQAPGKGLEWVS	GIWPSGGKTDYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	EGVIAVAGPYRD	WGQGTLVTVSS
807B-M0017-B06	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYEMG	WVRQAPGKGLEWVS	SISPSGGYTSYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0017-E05	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYQMA	WVRQAPGKGLEWVS	GISSSGGTTTYADSVKG	RFTISRDNSKNTLYLQMNSLSAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0018-C12	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	FYRMG	WVRQAPGKGLEWVS	SISSSGGLTDYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0018-E09	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYGMA	WVRQAPGKGLEWVS	YISPSGGGTSYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0018-G02	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	KYVMN	WVRQAPGKGLEWVS	SISSSGGQTSYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GERAAAGTQHYYYYYGMDV	WGQGTTVTVSS
807B-M0019-A04	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	YYDMW	WVRQAPGKGLEWVS	RIVSSGGWTMYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GRSLYYDFWSGYYPNTYYYYYMDV	WGKGTTVTVSS
807B-M0019-A10	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	YYDMS	WVRQAPGKGLEWVS	SIWSSGGNTMYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GGGFGVYHHYYDMDV	WGQGTTVTVSS
807B-M0019-C01	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYGMG	WVRQAPGKGLEWVS	YISSSGGHTKYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0019-F06	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYPMD	WVRQAPGKGLEWVS	SISPSGGFTQYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0019-G07	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	FYPMV	WVRQAPGKGLEWVS	WISSSGGTTSYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0020-D01	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYVMT	WVRQAPGKGLEWVS	GISSSGGMTEYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0020-F06	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	VYNMY	WVRQAPGKGLEWVS	SISPSGGFTTYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DTSGWYEEEDY	WGQGTLVTVSS
807B-M0020-F12	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	IYEMA	WVRQAPGKGLEWVS	SISPSGGWTKYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDH	WGQGTLVTVSS
807B-M0020-G01	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	FYYMS	WVRQAPGKGLEWVS	GISPSGGTTQYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0079-B09	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	FYAMQ	WVRQAPGKGLEWVS	YISSSGGHTHYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GAGALGY	WGQGTLVTVSS
807B-M0079-D10	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	NYRME	WVRQAPGKGLEWVS	SIWSSGGLTKQADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GLYR	WGQGTLVTVSS
807B-M0079-H01	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYDMD	WVRQAPGKGLEWVS	RIWPSGGSTHYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807B-M0079-H05	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	QYMMG	WVRQAPGKGLEWVS	SISSGGWTAYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	DGGTWDFDY	WGQGTLVTVSS

807B-M0080-A02	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYNMG	WVRQAPGKGLEWVS	SIGPSGGHTMY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0080-C04	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYSME	WVRQAPGKGLEWVS	SIVSSGGHTIYA DSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807B-M0080-F10	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	DYVMN	WVRQAPGKGLEWVS	SIYPSGGLTRY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	GAGALGY	WGQGTLVTVSS
807B-M0081-C03	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYWMG	WVRQAPGKGLEWVS	GISSSGGRTVY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	GHWGWFDP	WGQGTLVTVSS
807B-M0081-C05	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYNMD	WVRQAPGKGLEWVS	SIGPSGGPTKY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807B-M0081-D08	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	EYTML	WVRQAPGKGLEWVS	GIWPSGGPTFY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	ELDTAMAPPSDAFDI	WGQGTMVTVSS
807B-M0081-E08	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	MYNMY	WVRQAPGKGLEWVS	RIGSSGGMTDY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807B-M0081-F12	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYLMH	WVRQAPGKGLEWVS	SIVPSGGTTVY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	
807B-M0081-G04	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYSMV	WVRQAPGKGLEWVS	VISSSGGFTGY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0081-G11	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYDMV	WVRQAPGKGLEWVS	GIWPSGGFTNY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	HPVSSGFDY	WGQGTLVTVSS
807B-M0081-H03	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYSMV	WVRQAPGKGLEWVS	SIGPSGGMTRY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DQGITMVQGAMGY	WGQGTLVTVSS
807B-M0081-H07	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	FYTMV	WVRQAPGKGLEWVS	VISPSGGLTHY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0082-B07	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	YYSMT	WVRQAPGKGLEWVS	SISPSGGGTGY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0082-E01	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYGMS	WVRQAPGKGLEWVS	WISPSGGMTKY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0082-E08	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYDMD	WVRQAPGKGLEWVS	SISSSGGFTTYA DSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807B-M0082-H06	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYGMG	WVRQAPGKGLEWVS	YISSSGGLTIYA DSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0083-B10	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	YYAMG	WVRQAPGKGLEWVS	WISPSGGATHY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0083-E10	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	YYSMV	WVRQAPGKGLEWVS	WISSSGGSTNY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0083-E11	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	VYSMA	WVRQAPGKGLEWVS	GIWPSGGPTAY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	EDFWSGLEDV	WGKGTTVTVSS
807B-M0084-C03	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYDMM	WVRQAPGKGLEWVS	YIYSSGGSTRY ADSVKG	RFTISRDNSKKTLYLQMNSL RAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807B-M0084-C11	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	FYRMA	WVRQAPGKGLEWVS	VISPSGGHTYY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DFFTSYFDY	WGQGTLVTVSS
807B-M0084-E07	EVQLLESGGGLVQPG GSLRLSCAASGFTFS	WYRMA	WVRQAPGKGLEWVS	SISSSGGDTQY ADSVKG	RFTISRDNSKNTLYLQMNSL RAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS

807B-M0084-F03	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYVMD	WVRQAPGKGLEWVS	SISPSGGGTLYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLHYGSVLDF	WGQGTLVTVSS
807B-M0084-F08	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYGMA	WVRQAPGKGLEWVS	YISPSGGGTSYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0084-H05	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYTME	WVRQAPGKGLEWVS	GIYSSGGTTTYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807B-M0085-B12	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYGMV	WVRQAPGKGLEWVS	SISPSGGQTFYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807B-M0085-C01	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYTMD	WVRQAPGKGLEWVS	GISPSGGYTTYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	GTVLLWFGESGGHFDY	WGQGTPVTVSS
807B-M0085-E10	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYQMS	WVRQAPGKGLEWVS	GISSSGGSTQYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0085-G03	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYVMM	WVRQAPGKGLEWVS	SIVPSGGGTGYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	ENYGPDY	WGQGTLVTVSS
807B-M0085-G07	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYAMD	WVRQAPGKGLEWVS	SIVPSGGRTLYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807B-M0085-G08	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	FYGMG	WVRQAPGKGLEWVS	RIRPSGGMTSYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0086-C06	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYSMV	WVRQAPGKGLEWVS	WISSSGGFTKYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DLWFGEWDY	WGQGTLVTVSS
807B-M0086-D03	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYTMG	WVRQAPGKGLEWVS	SIVSSGGYTPYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	EPIWGYYYYGMDV	WGQGTTVTVSS
807B-M0086-E08	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	WYDMH	WVRQAPGKGLEWVS	WIVPSGGITEYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	QEVWQWPAQFDS	WGQGTLVTVSS
807B-M0086-G03	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	EYKMN	WVRQAPGKGLEWVS	YIYPSGGFTHYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	SVVGWGLDY	WGQGTLVTVSS

Table 16

Isolate Name	Enrichment		bCTD	VLDL	Peptide Mapping										koff	RU	Fab
	VH	CDR3			1	2	3	4	5	6	7	8	9	10	(e-3)		iHC
807B-M0011-C07	3	20	+												11.6	325	
807B-M0012-C09	12	12	+												11.7	156	
807B-M0012-D09	1	299	+												14.8	159	
807B-M0012-F10	1	1	+												5.5	50.5	
807B-M0012-F12	2	20	+												3.0	246	
807B-M0012-G05	15	299	+												7.9	157	
807B-M0013-A12	3	3	+												6.8	63.9	+
807B-M0013-B04	1	4	+												12.5	101	
807B-M0013-C03	1	299	+				+								38.4	177	
807B-M0013-F06	1	1	+				+					+			9.3	162	
807B-M0013-G05	1	20	+	+/-			+								17.5	136	
807B-M0014-D07	10	10	+									+			8.1	206	
807B-M0014-D09	13	13	+									+			5.2	80.4	
807B-M0014-E08	2	2	+				+								18.6	151	
807B-M0014-F07	1	2	+												8.6	205	
807B-M0016-C06	5	299	+												7.2	74.2	
807B-M0016-D01	1	299	+												8.5	74	
807B-M0016-D08	1	299	+				+								12.4	80.3	
807B-M0016-E01	1	20	+												8.0	465	
807B-M0016-F04	1	2	+												13.5	58.9	
807B-M0016-F05	15	299	+												9.0	69	
807B-M0016-F08	1	299	+												18.5	113	
807B-M0017-B05	1	1	+	+/-											26.1	248	
807B-M0017-B06	1	299	+												8.8	120	
807B-M0017-E05	1	299	+												4.7	71.1	
807B-M0018-C12	2	299	+												11.0	107	
807B-M0018-E09	3	299	+												10.5	66.8	
807B-M0018-G02	1	1	+									+			21.1	98.9	
807B-M0019-A04	1	1	+												5.4	31.1	
807B-M0019-A10	1	1	+												7.1	51	
807B-M0019-C01	1	299	+												10.6	80.1	
807B-M0019-F06	1	299	+												7.7	99.4	
807B-M0019-G07	1	299	+												5.2	31.2	
807B-M0020-D01	1	299	+												3.9	51.2	
807B-M0020-F06	1	1	+												4.9	53.2	
807B-M0020-F12	1	1	+												9.9	67.4	
807B-M0020-G01	1	299	+												12.7	68	
807B-M0079-B09	1	2	+	+/+							+				8.3	438	
807B-M0079-D10			+					+				+	+		4.3	100	+
807B-M0079-H01	1	20	+				+	+	+						2.5	256	
807B-M0079-H05	1	1	+												2.0	99.3	
807B-M0080-A02	1	299	+												2.5	86.8	
807B-M0080-C04	1	20	+												2.5	162	
807B-M0080-D06	1	1	+						+						2.4	72.7	
807B-M0080-F10	1	2	+							+					2.7	49.2	
807B-M0081-C03	1	1	+									+			2.2	84.6	
807B-M0081-C05	1	20	+												2.6	158	
807B-M0081-D08	1	1	+							+					4.0	55.1	

807B-M0081-E08	1	20	+												5.7	65.1	
807B-M0081-F12	249	299	+												6.1	128	+
807B-M0081-G04	1	299	+												2.6	64.1	
807B-M0081-G11	1	1	+							+					3.6	83.6	
807B-M0081-H03	1	1	+												9.7	346	+/-
807B-M0081-H07	1	299	+												2.3	56.4	
807B-M0082-B07	1	299	+	+/-											2.8	46.4	
807B-M0082-C11	1	1	+												4.0	244	
807B-M0082-E01	2	299	+						+	+					4.7	68.8	
807B-M0082-E08	1	20	+												3.0	154	
807B-M0082-F04	1	20	+												3.1	136	
807B-M0082-F11	1	299	+												6.1	32.5	
807B-M0082-H06	2	299	+					+							5.1	103	
807B-M0083-B10	1	299	+						+	+					2.9	45	
807B-M0083-E10	1	299	+						+						3.7	47.9	
807B-M0083-E11	3	4	+												5.1	34.6	+
807B-M0084-C03	1	20	+												2.0	53.9	
807B-M0084-C11	1	2	+												3.7	52.5	
807B-M0084-E07	1	299	+		+				+		+				4.4	46.7	
807B-M0084-F03	1	1	+												3.6	49.6	
807B-M0084-F08	3	299	+												5.8	51.5	
807B-M0084-H05	2	20	+	+											2.4	118	
807B-M0085-B12	1	20	+						+						7.9	95.3	
807B-M0085-C01	1	1	+												5.5	69.7	
807B-M0085-E01	1	20	+												2.8	49.5	
807B-M0085-E10	1	299	+		+	+			+	+		+	+	+	5.8	47.5	
807B-M0085-G03	1	1	+												2.2	52.8	
807B-M0085-G07	1	20	+												4.2	83.7	
807B-M0085-G08	1	299	+										+		3.8	42.2	
807B-M0086-C06	1	299	+												4.8	59.5	
807B-M0086-D03	1	20	+												3.7	159	
807B-M0086-E08	1	2	+												2.6	73	
807B-M0086-G03	2	2	+												3.7	43.8	

Table 17: Fab Binding Data to CTD and peptides

Isolate name	Strategy	IHC	CTD ELISA	peptide	group	Affinity Biacore (nM)						VLDL ELISA
						CTD	p1	p4	p6	p8	p9	
807B-M0001-B07	E22 P1	+/-	+	p1, p6	4	46	245		396			-
807B-M0004-A03	E22 P4	++	+	p4, p9	5	low RU*		98			low RU*	-
807B-M0004-A05	E22 P4	+/-	-	p4	1	no RU**		208				-
807B-M0004-C04	E22 P4	+/-	-	p4	1	no RU**		509				-
807B-M0004-C05	E22 P4	+/-	-	p4	1	no RU**		225				-
807B-M0004-F06	E22 P4	+/-	-	p4	1	no RU**		361				-
807B-M0004-F10	E22 P4	+/-	-	p4, p9	2	no RU**		104			low RU*	-
807B-M0004-H03	E22 P4	++	-	p4, p9	2	no RU**		200			low RU*	-
807B-M0009-C03	E22 P9, E24 P3	+/-	+	p4, p9	5	no RU**		728			163	-
807B-M0009-F06	E22 P9, E24 P3	+/-	-	p9	3	no RU**					172	-
807B-M0013-A12	E24	++	+	no	7	no RU**						-
807B-M0079-D10	E22&E23 P9, E24	++	+	p4, p8	6	59.17		low RU*		25.79		-

807B-M0081-F12	E24	+	+	no	8	low RU*						-
807B-M0081-H03	E24	+/-	+	no	9	10						-
807B-M0083-E11	E24	++	+	no	10	low RU*						-
807A-M0028-B02	E5	+	+	no								-
807A-M0026-F05	E5	+	+	p3, p8								-
807A-M0027-E11	E5	+	+	no								-

* low RU: RUs are low, affinity was not measured
** no RU: no binding observed

Table 18: IgG Binding Data to CTD and peptides

Isolate name	Strategy	IHC	CTD ELISA	peptide	Affinity Biacore (nM)							VLDL ELISA (O.D)†	ELISA		
					hCTD	mCTD	pCTD	p1	p4	p8	p9		hCTD	mCTD	pCTD
807B-M0001-B07	E22 P1	+	+	p1, p6	5	<h,pCTD	6	8.04				2.14	+++	++	+++
807B-M0004-A03	E22 P4	+++	+	(p1, p6) p4, p8, p9	3		3		0.17		low RU*	0.273	+	+	++
807B-M0004-A05	E22 P4	-	+	p4, p9	167	no RU**	no RU**		17.42			0.214	+/-	+/-	+/-
807B-M0004-C04	E22 P4	-	+/-	p4, p9	no RU**	no RU**	no RU**		8.68			0.242	+/-	+/-	+/-
807B-M0004-C05	E22 P4	-	-	p4, p9	no RU**	no RU**	no RU**		0.97			0.233	+/-	+/-	+/-
807B-M0004-F06	E22 P4	-			no RU**	no RU**	no RU**		6.72			0.248	+/-	+/-	+/-
807B-M0004-F10	E22 P4	(+)	+	p4, p8, p9	25	no RU**	23		1.00		13.74	1.11	++	+/-	+/-
807B-M0004-H03	E22 P4	+(+)	+	p4, p9, (p8)	45	no RU**	43		11.55		low RU*	0.309	++	+(+)	++
807B-M0009-C03	E22 P9, E24 P3	(+)	+	p4, p9	23	no RU**	4		1.00		0.27	2.528	+++	+++	+++
807B-M0009-F06	E22 P9, E24 P3	++	-	p4, p9	34	234	28		no RU**		10.6	1.202	+/-	+/-	+/-
807B-M0013-A12	E24	+++	+	(p3, p9), p4, p8	27	23	26					0.462	++	+/-	+/-
807B-M0079-D10	E22&E23 P9, E24	(+)	+	p4, p8	1	2	1		43.64	1.05		0.2	+++	+++	+++
807B-M0081-F12	E24		+	(p9)	15	16	19		no RU**			0.285	++	+	+
807B-M0081-H03	E24	(+)	+	(p7)	9	18	7		no RU**			2.88	+++	+++	+++
807B-M0083-E11	E24	-	+	no	12	64	25		no RU**			0.221	+	-	-
807A-M0028-B02	E5	+	+	P4	9	7	5					2.679			
807A-M0026-F05	E5	+													-
807A-M0027-E11	E5	+													-

* low RU: RUs are low, affinity was not measured ** no RU: no binding observed

† VLDL ELISA: VLDL is coated, 5µg/ml of appropriate hIgG is added O.D. of 2x background is 0.420

Table 19: Amino acid sequences of the VL chains of the Germline-corrected antibodies

Initial Name	LV-FR1	LV-CDR1	LV-FR2	LV-CDR2	LV-FR3	LV-CDR3	LV-FR4
807A-M0028-B02.1	DIQMTQSPSSLSA SVGDRVTITC	RTSQDIRNHLG	WFQQKPGKAP QRLIR	EASILQS	GVPSTFYGSGYGREFTLTIS SLQPEDFATYYC	LQYDSFPYT	FGQGTKLEIK
807A-M0028-B02.2	DIQMTQSPSSLSA SVGDRVTITC	RTSQDIRNHLG	WYQQKPGKAP KRLIY	EASILQS	GVPSRFSGSGSGTEFTLTIS SLQPEDFATYYC	LQYDSFPYT	FGQGTKLEIK
807B-M0004-H03.1	DIQMTQSPSSLSA SVGDRVTITC	QASQNIDNYLN	WYQQKPGKAP KLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTIS SLQPEDFATYYC	QQSYSTPRT	FGQGTKVEIK
807B-M0009-F06.1	DIVMTQSPLSLPV TPGEPASISC	KSSQSLLHSNGYNYLD	WYLQKPGQSP QLLIY	LGSNRAS	GVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYC	MQALQTIT	FGQGTRLEIK
807B-M0004-A03.1	QSVLTQPPSASG TPGQRVTISC	SGSSSNIGSNTVN	WYQQLPGTAP KLLIY	NNNQRPS	GVPDRFSGSKSGTSASLAIS GLQSEDEADYYC	AAWHDGLNGPV	FGGGTKLTVL
807B-M0079-D10.1	SYELTQPPSVSVS PGQTASITC	AGDELGNKYAS	WYQQKPGQSP VLVIY	QDRKRPS	GIPERFSGSNSGNTATLTIS GTQAMDEADYYC	QSWDSSSVI	FGGGTKLTVL

Table 20: Amino acid sequences of the CL chains of the Germline-corrected antibodies

Name	Germ line	Sequence of constant region of light chain
807A-M0028-B02.1	Kappa	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
807A-M0028-B02.2	Kappa	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
807B-M0004-A03.1	Lambda	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSY SCQVTHEGSTVEKTVAPTECS
807B-M0079-D10.1	Lambda	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSY SCQVTHEGSTVEKTVAPTECS

Table 21: Description of SEQ ID NOS: 539-548

Antibody	VL chain sequence	CL chain sequence
807A-M0028-B02.1	SEQ ID NO: 518	SEQ ID NO: 519
807A-M0028-B02.2	SEQ ID NO: 520	SEQ ID NO: 521
807B-M0004-H03.1	SEQ ID NO: 524	-
807B-M0009-F06.1	SEQ ID NO: 525	-
807B-M0004-A03.1	SEQ ID NO: 522	SEQ ID NO: 523
807B-M0079-D10.1	SEQ ID NO: 526	SEQ ID NO: 527

Table 22: IgG Binding Data to CTD and peptides

Isolate name	Strategy	IHC[2]	CTD ELISA	peptide	Affinity Biacore (nM)							VLDL ELISA[5]	ELISA[6]		
					hCTD	mCTD	pCTD	p1	p4	p8	p9		hCTD	mCTD	pCTD
807B-M0001-B07	E22 P1	+	+	p1, p6	5	<h,pCTD	6	8.04				++	+++	++	+++
807B-M0004-A03	E22 P4	+++	+	(p1, p6) p4, p8, p9	3		3		0.17		low RU[4]	++	+++	++	+
807B-M0004-A05	E22 P4	-	+	p4, p9	167	no RU[3]	no RU[3]		17.42			-	+/-	+/-	+/-
807B-M0004-C04	E22 P4	-	+/-	p4, p9	no RU[3]	no RU[3]	no RU[3]		8.68			-	+/-	+/-	+/-
807B-M0004-C05	E22 P4	-	-	p4, p9	no RU[3]	no RU[3]	no RU[3]		0.97			-	+/-	+/-	+/-
807B-M0004-F06	E22 P4	-			no RU[3]	no RU[3]	no RU[3]		6.72			-	+/-	+/-	+/-
807B-M0004-F10	E22 P4	(+)	+	p4, p8, p9	25	no RU[3]	23		1.00		13.74	+	++	+/-	+/-
807B-M0004-H03	E22 P4	+(+)	+	p4, p9, (p8)	45	no RU[3]	43		11.55		low RU[4]	++	+	-	-
807B-M0009-C03	E22 P9, E24 P3	(+)	+	p4, p9	23	no RU[3]	4		1.00		0.27	++	+++	+++	+++
807B-M0009-F06	E22 P9, E24 P3	++	-	p4, p9	34	234	28		no RU[3]		10.6	+++	+++	+	+
807B-M0013-A12	E24	+++	+	(p3, p9), p4, p8	27	23	26					++	++	+/-	+/-
807B-M0079-D10	E22&E23 P9, E24	(+)	+	p4, p8	1	2	1		43.64	1.05		+++	+++	+++	+++
807B-M0081-F12	E24	[1]n.d	+	(p9)	15	16	19		no RU[3]			+	++	+	+
807B-M0081-H03	E24	(+)	+	(p7)	9	18	7		no RU[3]			+++	+++	+++	+++
807B-M0083-E11	E24	-	+	no	12	64	25		no RU[3]			-	+	-	-

807A-M0028-B02	E5	+	+	P4	9	7	5					+++	+++	+++	+++
807A-M0026-F05	E5	(+)										+++	+++	[1]n.d	[1]n.d
807A-M0027-E11	E5	+										+++	+++	[1]n.d	[1]n.d

[1]n.d.: Not done
[2]IHC: tissue from human AD cortex used. 1 µg/ml of appropriate hIgG added
[3]no RU: no binding observed
[4]low RU: RUs are low, affinity was not measured
[5]VLDL ELISA: human VLDL coated 0.0003-100 µg/ml of appropriate hIgG tested for binding
[6]ELISA: human, mouse or primate CTD coated, 0.0003-100 µg/ml of appropriate hIgG tested for binding

Table 23: Binding of germ line corrected IgG to CTD and tissue

Isolate name	Peptide	Affinity (Biacore (nM))	VLDL ELISA[2]	IHC[3] (tissue)	ELISA[4]			*In vitro* phagocytosis EC50± SEM (ng/ml)
					hCTD	mCTD	pCTD	
807A-M0028-B02	P4	10.8	+++	++	+++	+++	+++	34+/- 15
807A-M0028-B02.1	n.d.[1]	13	++	++	+++	+++	++	67 +/- 38
807A-M0028-B02.2	n.d.[1]		+++	++	+	+	+	27 +/- 7
807B-M0004-H03	p4, p9, (p8)	12.5	++	++	+	-	+	53 +/- 25
807B-M0004-H03.1	n.d.[1]	17.7	-	+++	+++	-	++	22 +/- 16
807B-M0009-F06	p4, p9	14.8	+++	++	+++	+	++	23 +/- 9
807B-M0009-F06.1	n.d.[1]	very low binding	-	+	-	-	-	42 +/- 17
807B-M0004-A03	(p1, p6) p4, p8, p9	12.2	++	++	+++	+	++	205 +/- 81
807B-M0004-A03.1	n.d.[1]	24.4	-	+[3*]	+++	+	+	78 +/- 43
807B-M0079-D10	p4, p8	n.d.[1]	+++	++	+++	+++	+++	7 +/- 1.4
807B-M0079-D10.1	n.d.[1]	n.d.[1]	++	++	+++	+++	+++	19 +/- 15

[1] n.d.: Not done.

[2] VLDL ELISA: human VLDL is coated 0.0003-100 μg/ml of appropriate hIgG is tested for binding

[3] IHC: Brain tissue sections from APP/PS1 mouse was used. 1,5-1.8 μg/ml of appropriate hIgG is added,

[3*] reactivity to mCTD lower than to hCTD

[4] CTD ELISA: human, mouse or primate CTD is coated, 0.0003-100 μg/ml of appropriate hIgG is tested for binding

Table 24: 807B-M0004-A03 = 39 clones selected for Fab production

AminoAc	1(WT=S)	2(WT=I)	3(WT=A)	4(WT=A)	5(WT=A)	6(WT=G)	7(WT=T)	8(WT=D)	9(WT=Y)
A			88%	65%	60%				
D					9%			98%	2%
E				7%				2%	
G	2%		5%	2%		84%			
H							2%		21%
I		98%					2%		
K							5%		
L									2%
M		2%							
N				5%			10%		
P					5%				
Q			2%						
R	5%					16%	2%		5%
S	91%		5%	14%	19%				
T	2%			2%	7%		79%		
V				5%					
Y									70%

Table 25: 807B-M0004-H03 = 54 clones selected for Fab production: part 1

AminoAc	1(WT=E)	2(WT=G)	3(WT=S)	4(WT=A)	5(WT=G)	6(WT=V)	7(WT=V)	8(WT=K)	9(WT=G)	10(WT=P)
A	2%	2%	5%	82%	9%	5%			4%	5%
D	4%						2%			
E	95%				2%	4%				
F						4%	4%		2%	
G		73%		2%	46%				73%	
H										2%
I							7%	7%		
K						2%		84%		
L			5%			9%	9%			5%
M			2%			4%	2%			
N								4%		
P			5%					2%		77%
Q								2%		2%
R		18%	2%	9%	38%	4%		2%	11%	5%
S		4%	75%							4%
T						2%				
V		4%		7%	4%	66%	77%			
W			4%						9%	
Y										
Stop			2%							

Table 26: 807B-M0004-H03 = 54 clones selected for Fab production: part 2

AminoAc	11(WT=A)	12(WT=R)	13(WT=Y)	14(WT=Y)	15(WT=Y)	16(WT=Y)	17(WT=Y)	18(WT=M)	19(WT=D)	20(WT=V)
A	73%								4%	4%
C			2%							
D	2%						2%		77%	
E							2%		4%	
F			2%	4%		5%				5%
G	5%	2%							2%	2%
H			7%		7%	2%			4%	
I								5%		7%
K		4%								
L	2%	2%						2%		9%
M								93%		
N			2%	4%	4%		4%		5%	
P	4%	2%								2%
Q		4%					5%			
R	2%	83%								
S	5%				4%		5%		2%	
T	5%									
V	4%								2%	71%
Y		4%	88%	92%	85%	93%	82%		2%	

Table 27: 807B-M0079-D10 = 33 clones selected for Fab production

AminoAc	1(WT=G)	2(WT=L)	3(WT=Y)	4(WT=R)
A	11%		6%	
D	3%			
F			9%	
G	69%			3%
H			11%	9%
I		3%		
K				3%
L		86%	3%	17%
M		6%		
N			6%	
P				3%
Q		3%		9%
R				43%
S	14%		6%	3%
T	3%			
V		3%		3%
W				3%
Y			57%	6%
Stop			2%	

Table 28: 807A-M0028-B02-CTD = 60 clones selected for Fab production

AminoAc	1(WT=S)	2(WT=V)	3(WT=L)	4(WT=L)	5(WT=D)	6(WT=Y)
A	18%	2%			2%	2%
D		2%			92%	
E					2%	2%
F	2%			19%		3%
G	19%	3%				
H			3%	23%	2%	11%
I		23%	2%	2%		2%
K						16%
L		2%	87%	52%		2%
M			3%			
N		2%				11%
P	2%		2%	3%		3%
Q			2%			3%
R				2%		3%
S	57%					8%
T	2%	2%	2%			3%
V		66.50%				
Y	2%				2%	29%

Table 29: 807A–M0028-B02-fibrils = 12 clones selected for Fab production

AminoAc	1(WT=S)	2(WT=V)	3(WT=L)	4(WT=L)	5(WT=D)	6(WT=Y)
A	33%					
D					100%	
F				25%		
G						
H			8%	17%		17%
I						
K		8%				
L			92%	58%		
N						17%
Q						8%
R						8%
S	67%					17%
V		92%				
Y						33%

Table 30: 807B-M0009-F06 = 24 clones selected for Fab production

AminoAc	1(WT=V)	2(WT=G)	3(WT=M)	4(WT=S)	5(WT=T)	6(WT=Y)	7(WT=A)	8(WT=F)	9(WT=D)	10(WT=I)
A		8%		8%			24%			
D									96%	
E									4%	
F				4%				96%		4%
G		92%					60%			
H					4%					
I			20%							56%
K			4%							14%
L			12%	4%				4%		16%
M			60%							4%
N					12%					
P				8%						
S				60%	8%		16%			
T				12%	76%					8%
V	100%		4%							8%
Y				4%		100%				

Table 31: Biacore analysis of 807B-M0004-A03, original clone and variants

Clone	HV-CDR3	BC	koff	kon	KD	Ranking	
		nM	1/s	1/Ms	nM	koff	KD
* 807B-M0004-A03/WT	SIAAAGTDY	234	0.0294	3.18E+05	9.26E-08	27	13
807B-M0004-A03/WT	SIAAAGTDY	232	0.0275	2.54E+05	1.08E-07	23	16
807B-M0004-A03/M0117-A04	SIAADGIDY	181	0.0341	ND	3.07E+03	33	41
807B-M0004-A03/M0117-A12	SIAATRTDY	239	0.0215	1.47E+05	1.46E-07	10	28
807B-M0004-A03/M0117-B03	SIAAARTEY	192	0.0262	3.29E+04	7.97E-07	21	36
807B-M0004-A03/M0117-B04	SIAPSGTDY	210	0.132	6.22E-04	212	43	40
807B-M0004-A03/M0117-B05	SIAPAGTDH	224	0.0297	2.45E+05	1.21E-07	28	22
807B-M0004-A03/M0117-B11	SIAEAGTDY	245	0.0464	3.02E+05	1.54E-07	35	29
807B-M0004-A03/M0117-C04	SIAVAGTDY	193	0.0902	1.25	0.072	41	38
807B-M0004-A03/M0117-C07	SIAGAGNDY	186	0.0306	2.40E+05	1.28E-07	30	23
807B-M0004-A03/M0117-C09	SIAAAGTDH	280	0.0188	2.53E+05	7.44E-08	6	10
807B-M0004-A03/M0117-C11	SIGAAGTDY	263	0.0767	0.0967	0.788	39	39
807B-M0004-A03/M0117-C12	SIAASGTDY	134	0.0284	3.95E+05	7.22E-08	26	8
* 807B-M0004-A03/M0117-D03	SIAAARTDY	199	0.0128	9.45E+04	1.34E-07	3	25
807B-M0004-A03/M0117-E06	SIQAAGTDH	123	0.0247	1.96E-05	4.21E+03	15	42
807B-M0004-A03/M0117-E12	SIASPGTDY	228	0.0266	1.95E+05	1.37E-07	22	27
* 807B-M0004-A03/M0117-F05	SIASAGTDH	290	0.0139	2.22E+05	6.13E-08	4	4
807B-M0004-A03/M0117-F11	GISTSGTDD	284	0.0243	2.08E+05	1.15E-07	14	19
807B-M0004-A03/M0117-F12	SIAVAGTDY	235	0.0906	1.82E-05	4.96E+03	42	43
* 807B-M0004-A03/M0117-G01	SIASARTDS	256	0.0103	1.66E+05	6.07E-08	1	3
807B-M0004-A03/M0117-G03	SIAAPGTDY	220	0.011	1.92E+04	4.84E-07	2	35
807B-M0004-A03/M0117-G04	RIAASGTDY	92	0.049	4.42E+05	1.20E-07	36	21
807B-M0004-A03/M0117-G05	SIAATGKDH	280	0.0815	9.94E+04	8.24E-07	40	37

807B-M0004-A03/M0117-G07	SIAAAGSDS	471	0.0283	1.35E+05	2.23E-07	25	33
807B-M0004-A03/M0117-H06	SIGASRTDY	336	0.0502	3.42E+05	1.68E-07	37	31
807B-M0004-A03/M0117-H11	SIASAGTDL	217	0.0205	2.87E+05	6.97E-08	8	6
807B-M0004-A03/M0118-A03	SIAAAGNDY	167	0.0254	3.51E+05	7.12E-08	17	7
* 807B-M0004-A03/M0118-B09	SIAADRTDY	252	0.0231	4.78E+05	4.70E-08	11	1
807B-M0004-A03/M0118-B11	SIAESGTDY	203	0.0298	3.34E+05	9.06E-08	29	12
807B-M0004-A03/M0118-C04	SIASSGTDH	213	0.0205	3.64E+05	5.47E-08	9	2
807B-M0004-A03/M0118-D02	RMAAAGTDY	225	0.0235	9.81E+04	2.42E-07	12	34
807B-M0004-A03/M0118-D03	SIAAAGKDY	281	0.0306	2.90E+05	1.33E-07	31	24
807B-M0004-A03/M0118-D07	SIAATGTDI	263	0.028	2.54E+05	1.19E-07	24	20
807B-M0004-A03/M0118-E10	SIAAAGNDH	179	0.0236	3.19E+05	7.41E-08	13	9
807B-M0004-A03/M0118-E12	SIASAGTDY	267	0.0312	2.52E+05	1.55E-07	32	30
* 807B-M0004-A03/M0118-F03	SIAASRTDY	230	0.0158	2.36E+05	6.56E-08	5	5
807B-M0004-A03/M0118-F06	SIAAAGTDH	372	0.0194	2.38E+05	7.97E-08	7	11
807B-M0004-A03/M0118-F09	SIAEAGTDY	245	0.0456	2.92E+05	1.78E-07	34	32
807B-M0004-A03/M0118-F12	SISAAGTDY	278	0.0249	1.78E+05	1.37E-07	16	26
807B-M0004-A03/M0118-G03	SIAADGTDY	208	0.0669	6.16E+05	1.14E-07	38	18
807B-M0004-A03/M0118-G05	TIAAAGTDY	267	0.0259	2.42E+05	1.13E-07	20	17
807B-M0004-A03/M0118-G08	SIAAAGHDH	336	0.0256	2.70E+05	1.00E-07	18	14
807B-M0004-A03/M0118-H01	SIAAAGNDY	276	0.0258	2.69E+05	1.00E-07	19	15

* = selected clone

BC = Biacore

Table 32: Biacore analysis of 807B-M0004-H03, original clone and variants

Clone	HV-CDR3	Biacore	koff	kon	KD	Ranking	
		nM	1/s	1/Ms	nM	koff	KD
* 807B-M0004-H03/WT	EGSAGVVKGPARYYYYYMDV	703	1.18E-03	4.37E+03	2.56E-07	1	16
807B-M0004-H03/M0119-A07	ERSAGVLKGPAWYYYYYMDV	262	5.94E-03	1.48E+04	1.67E-07	36	7
807B-M0004-H03/M0119-A08	EGSAAFVKGPARYYYYDMDI	375	2.66E-01	1.24E+04	4.64E-07	55	30
* 807B-M0004-H03/M0119-B05	EGSSGVVKGPARYYYYYMDA	371	1.53E-03	6.03E+03	1.59E-07	4	5
807B-M0004-H03/M0119-B06	EGSVGAVKGRARYYYYYMNV	729	2.62E-03	3.45E+03	4.00E-07	16	26
807B-M0004-H03/M0119-B07	EGSAGVFKGPARYYYYYMDV	486	7.20E-03	1.36E+04	3.98E-07	40	24
* 807B-M0004-H03/M0119-C05	ERSVAVFKARPRHYYYYMDV	696	1.69E-03	1.09E+04	1.46E-07	5	3
807B-M0004-H03/M0119-C08	EGSAGVDIGPARYYYYYMNV	453	7.24E-03	1.46E+04	4.72E-07	41	32
807B-M0004-H03/M0119-C11	EGSAAVVKAPAKYYYYYMEV	347	7.39E-03	1.47E+04	4.70E-07	43	31
807B-M0004-H03/M0119-E01	EGSVGVVKGPARYYHYQIDV	541	3.03E-03	7.69E+03	3.29E-07	19	21
807B-M0004-H03/M0119-E04	ESSARVVKGLARYYNYYMHV	484	5.34E-03	5.43E+03	6.00E-07	31	38
807B-M0004-H03/M0119-E07	ERPSRVVKGPTRYYYYYMDV	577	5.51E-03	4.33E-03	7.70E-01	32	52
807B-M0004-H03/M0119-E11	EVSARVVKCPARYYYYYMDV	614	9.69E-03	7.74E-06	1.19E+03	45	54
807B-M0004-H03/M0119-F01	EGSAGVIKGPARYYYFYMGV	389	1.85E-01	1.38E+04	7.14E-07	54	41
* 807B-M0004-H03/M0119-F04	EGSARVIKGPARYYYYEMDV	675	3.99E-03	2.68E+04	1.44E-07	29	2
807B-M0004-H03/M0119-F05	ERSVGVVIGHARYFYYYMDV	472	1.72E-03	8.05E+03	1.99E-07	6	8
807B-M0004-H03/M0119-F09	EGPAGVVKGRARYYSYNMSV	246	1.13E-01	8.96E+05	1.10E-07	52	1
807B-M0004-H03/M0119-F10	ESSARVVNGPAWYYYYYMDA	261	5.71E-03	8.52E+03	6.47E-07	35	39
807B-M0004-H03/M0119-F11	EGSSRAVKGAPRYYYYYMDV	1027	3.41E-03	1.52E+03	1.68E-06	24	45
807B-M0004-H03/M0119-F12	EVSGGVVKGPARYYYYYMAL	862	2.57E-02	6.16E+03	3.93E-06	48	47
807B-M0004-H03/M0119-G08	EGqARRVKGQARYYYYYMDV	270	3.33E-03	3.29E+03	6.81E-07	23	40
807B-M0004-H03/M0119-G10	EGSAGLVKGPARYYYYYMDV	514	1.76E-03	4.94E+03	2.95E-07	7	19
807B-M0004-H03/M0119-G12	ERSAGVVKGPSRNYYYYMDV	728	3.73E-03	1.56E+04	2.36E-07	27	14
807B-M0004-H03/M0119-H01	EGSARRVKRPGRYYYYQMDV	912	1.06E-02	3.66E+04	2.84E-07	46	18
807B-M0004-H03/M0119-H03	EGSARMLKGPARCYYYYMDV	315	7.36E-03	1.55E+04	4.38E-07	42	28
807B-M0004-H03/M0119-H08	EGMAGVVKFPARHNYHYMDV	881	4.58E-02	8.86E+04	5.76E-07	49	37
* 807B-M0004-H03/M0119-H09	DGSARVVKGPRRYYYYYIDV	351	1.40E-03	7.92E+03	1.50E-07	2	4
807B-M0004-H03/M0119-H11	ERPAGLVKGPARYYSYYMDV	1205	2.21E-03	6.55E+03	3.11E-07	12	20

807B-M0004-H03/M0120-A03	EGSARMVKGAARYYYYYMDV	1109	1.62E-02	6.25E+04	2.50E-07	47	15
807B-M0004-H03/M0120-A07	EGSAGTIKWLVRYYNFYMDV	707	3.24E-03	6.77E+03	4.48E-07	21	29
* 807B-M0004-H03/M0120-B05	EGSARVVKGPARYFYYYMDL	740	1.40E-03	4.70E+03	2.34E-07	3	13
807B-M0004-H03/M0120-B06	EGSARVVKGPDRYYYYYMAP	309	5.62E-03	8.84E-03	4.82E-01	33	51
807B-M0004-H03/M0120-B09	EGSAGKVIGPAPHYYYYMDV	868	6.90E-03	2.05E+04	3.34E-07	39	22
807B-M0004-H03/M0120-B11	EGRARVLKGLARYYHYYMDF	1023	3.01E-03	3.50E+02	5.42E-06	18	49
807B-M0004-H03/M0120-C02	EGSARFVKGPARYYYYYMDI	779	3.74E-03	5.68E+02	4.10E-06	28	48
807B-M0004-H03/M0120-C06	EGSSRLVQWPARYYYYSMDV	747	3.67E-03	5.96E+03	5.18E-07	26	35
807B-M0004-H03/M0120-C07	ERSAGVMKGPTLYYYYYMDV	641	8.85E-03	6.51E+03	1.17E-06	44	44
807B-M0004-H03/M0120-C12	EGSAGVVNRSSRYNYYYLDV	959	4.94E-02	ND	ND	50	-
807B-M0004-H03/M0120-D04	EGSSVEVKGPARYYHYYMDV	652	1.85E-03	6.71E+03	2.07E-07	10	9
807B-M0004-H03/M0120-D05	EGSAGVVKGPTRYYYYSMDV	921	2.28E-03	3.84E+03	4.81E-07	15	33
807B-M0004-H03/M0120-E02	EGSAVVVKRSARYYYYYMNF	827	6.72E-03	6.74E+03	8.20E-07	38	42
807B-M0004-H03/M0120-E04	ERSARLLKGPLRYYYYYMDV	817	1.86E-03	2.35E+03	3.70E-07	11	23
807B-M0004-H03/M0120-E06	ERAARAVKGPSRYYYYYMHV	1110	1.15E-01	3.93E-03	3.76E+00	53	53
807B-M0004-H03/M0120-F03	EGLAGVVKRPARFYYYYMDV	873	3.26E-03	1.09E+04	2.80E-07	22	17
807B-M0004-H03/M0120-F04	EGSARVVIWPAQYYYYYMDF	434	3.53E-03	5.48E+03	4.89E-07	25	34
807B-M0004-H03/M0120-F06	EGSARVVKGPARYYYYSMVV	850	2.94E-03	4.44E+03	4.17E-07	17	27
807B-M0004-H03/M0120-F07	ERSAAVVKWPVRYYYYYMDL	611	4.92E-03	1.26E+03	2.41E-06	30	46
807B-M0004-H03/M0120-G02	EGWAALVKGPGRYYYYQMYV	749	3.19E-01	4.67E+02	2.80E-05	56	50
807B-M0004-H03/M0120-G03	EGSAGVLKGPAKYYYYYMDI	755	1.77E-03	6.24E+03	2.33E-07	8	12
807B-M0004-H03/M0120-G04	EGSARVVKGPARYYYYYMDV	956	1.81E-03	1.09E+04	1.64E-07	9	6
807B-M0004-H03/M0120-G10	EGSAGVVKGPARHYYYYMDI	755	2.28E-03	9.65E+03	2.30E-07	14	11
807B-M0004-H03/M0120-G12	DGLAEEVKGPAQYYYYYIDG	782	7.80E-02	7.72E+04	1.02E-06	51	43
807B-M0004-H03/M0120-H03	EAAAGVVKGPARYYYFNMEV	1114	2.27E-03	4.87E+03	3.99E-07	13	25
807B-M0004-H03/M0120-H04	EGAARAVRRPAGYYHYYMDL	916	3.22E-03	1.46E+04	2.16E-07	20	10
807B-M0004-H03/M0120-H06	QGWAGVVKWPARYYYYYMDV	3	5.71E-03	ND	ND	34	-
807B-M0004-H03/M0120-H10	EGLAGVIPRAARYYYYYMDL	800	6.12E-03	1.06E+04	5.45E-07	37	36

* = *selected clones*

Table 33: Biacore analysis of 807B-M0009-F06, original clone and variants

Clone	HV-CDR3	Biacore	koff	kon	KD	Ranking	
		nM	1/s	1/Ms	nM	koff	KD
807B-M0009-F06/WT	VGMSTYAFDI	243	9.70E-02	0.043	2.26	23	25
807B-M0009-F06/M0127-A01	VGMSTYGLEI	471	2.79E-02	2.14E+04	1.26E-06	17	18
*807B-M0009-F06/M0127-B07	VGMSTYGFDK	418	8.83E-03	5.62E+04	1.56E-07	2	8
807B-M0009-F06/M0127-B08	VGMTTYAFDV	390	8.59E-02	0.0434	1.85	20	22
*807B-M0009-F06/M0127-C10	VGISTYGFDL	78	1.53E-02	1.74E+05	8.68E-08	10	2
807B-M0009-F06/M0127-D01	VGISTYGFDI	324	1.45E-02	5.56E+04	2.57E-07	7	15
*807B-M0009-F06/M0127-D05	VGMATYGFDI	346	9.45E-03	6.97E+04	1.34E-07	3	5
807B-M0009-F06/M0127-E03	VGISTYGFDV	240	1.49E-02	1.09E+05	1.35E-07	9	6
807B-M0009-F06/M0127-E10	VGMSTYGFDI	289	1.05E-02	7.43E+04	1.40E-07	5	7
807B-M0009-F06/M0127-E11	VGIPTYSFDI	146	2.24E-02	2.61E+04	8.57E-07	14	17
807B-M0009-F06/M0127-F07	VGLATYSFDL	203	1.02E-01	0.0628	1.62	25	20
*807B-M0009-F06/M0127-F09	VGMYNYGFDI	221	1.02E-02	1.09E+05	9.38E-08	4	3
807B-M0009-F06/M0127-F11	VGMSTYSFDT	271	1.01E-01	0.0453	2.23	24	24

807B-M0009-F06/M0127-G02	VGVSTYGFDI	39	1.59E-02	1.47E+05	1.08E-07	11	4
807B-M0009-F06/M0127-H04	VGMFTYAFDT	201	7.87E-02	0.0435	1.81	19	21
807B-M0009-F06/M0127-H05	VGKSTYGFDI	305	1.79E-02	8.37E+04	2.14E-07	12	13
807B-M0009-F06/M0128-C01	VAMTTYGFDL	253	2.41E-02	3.70E+04	6.52E-07	16	16
807B-M0009-F06/M0128-D09	VGISSYGFDI	316	1.46E-02	7.76E+04	1.88E-07	8	11
807B-M0009-F06/M0128-D12	VAMSNYGFDL	159	2.38E-02	1.13E+05	2.10E-07	15	12
807B-M0009-F06/M0128-F02	VGMTHYAFDI	148	1.98E-02	7.91E+04	2.50E-07	13	14
807B-M0009-F06/M0128-F08	VGMLTYAFDI	120	7.71E-02	0.0765	1.01	18	19
807B-M0009-F06/M0128-G09	VGLPSYSFDI	115	8.98E-02	1.24E+05	1.72E-07	22	10
*807B-M0009-F06/M0128-H01	VGMSNYGFDF	116	7.78E-03	1.62E+05	4.79E-08	1	1
807B-M0009-F06/M0128-H07	VGMSTYAFDM	211	8.98E-02	0.048	1.87	21	23
807B-M0009-F06/M0128-H11	VGLSTYGFDI	295	1.31E-02	8.09E+04	1.62E-07	6	9

* = selected clones

Table 34: Biacore screening of 807B-M0079-D10, original clone and variants

Clone	HV-CDR3	koff
		1/s
* 807B-M0079-D10/WT	GLYR	6.02E-03
807B-M0079-D10/M0121-A01	GLHR	1.29E-02
807B-M0079-D10/M0121-A02	GLYG	1.95E-02
807B-M0079-D10/M0121-A06	GLYH	1.47E-02
807B-M0079-D10/M0121-A08	GLHL	2.14E-02
807B-M0079-D10/M0121-A11	GIYR	1.43E-02
807B-M0079-D10/M0121-A12	ALAR	1.65E-02
807B-M0079-D10/M0121-B04	GLFR	1.07E-02
807B-M0079-D10/M0121-B05	SLYQ	2.31E-02
807B-M0079-D10/M0121-B12	GLLL	1.71E-02
807B-M0079-D10/M0121-C01	GQYR	1.93E-02
807B-M0079-D10/M0121-C03	GLAR	2.24E-02
807B-M0079-D10/M0121-D01	GLYQ	1.34E-02
807B-M0079-D10/M0121-D05	GLYP	5.93E-03
807B-M0079-D10/M0121-D06	GMYR	1.62E-02
807B-M0079-D10/M0121-E02	ALYS	5.34E-03
807B-M0079-D10/M0121-F02	GLSR	8.02E-03
807B-M0079-D10/M0121-F05	SLYL	1.84E-02
807B-M0079-D10/M0121-F06	GLYL	1.11E-02
807B-M0079-D10/M0121-F11	GMYV	5.92E-03
807B-M0079-D10/M0121-G03	SLYR	1.22E-02
807B-M0079-D10/M0121-G10	ALYR	1.03E-02
807B-M0079-D10/M0121-H04	SLYH	2.03E-02
807B-M0079-D10/M0121-H05	GLYY	1.70E-02
807B-M0079-D10/M0122-A01	DLYR	1.65E-02
807B-M0079-D10/M0122-B03	TLHR	2.58E-02
807B-M0079-D10/M0122-D01	GLHH	2.11E-02
807B-M0079-D10/M0122-D03	GLNR	1.29E-02
807B-M0079-D10/M0122-D05	GLSQ	1.43E-02
807B-M0079-D10/M0122-E06	GLqR	2.59E-02
807B-M0079-D10/M0122-F09	GLFY	8.76E-03
807B-M0079-D10/M0122-F11	GLNL	1.21E-02
807B-M0079-D10/M0122-G07	SLFK	2.17E-02
807B-M0079-D10/M0122-G12	ALYW	6.11E-03
807B-M0079-D10/M0122-H11	GVYL	1.32E-02

* = selected clones

Table 35: Biacore screening of 807A-M0028-B02, original clone and variants

Clone	HV-CDR3	BC	koff	kon	KD	Ranking	
		nM	1/s	1/Ms	nM	koff	KD
* 807A-M0028-B02/WT	SVLLDY	592	1.48E-02	1.73E+04	869	72	61
807A-M0028-B02/M0123-A04	SVQLYP	120	5.05E-02	0.0776	660000000	74	73
807A-M0028-B02/M0123-A05	SVLHDK	958	8.85E-03	1.16E+04	768	18	57
807A-M0028-B02/M0123-A06	FALLDY	247	7.82E-03	2.20E+04	358	13	28
807A-M0028-B02/M0123-A07	SVLFDK	548	6.97E-03	1.96E+04	358	10	27
807A-M0028-B02/M0123-A09	TLLLDs	345	1.47E-02	5.85E-03	2530000000	71	74
807A-M0028-B02/M0123-A10	GVLLDL	385	9.74E-03	2.13E+04	462	27	40
807A-M0028-B02/M0123-A11	SVLFDY	398	1.30E-02	1.29E+04	1020	66	67
807A-M0028-B02/M0123-A12	SILFDY	449	1.02E-02	1.36E+04	757	33	56
807A-M0028-B02/M0123-B01	SVLLDQ	711	9.49E-03	8.59E+03	1110	25	70
807A-M0028-B02/M0123-B03	SNLHDQ	199	1.21E-02	1.12E+04	1080	55	69
807A-M0028-B02/M0123-B06	AILLNY	207	7.43E-03	2.89E+04	257	12	15
807A-M0028-B02/M0123-B08	AVLLDH	471	1.03E-02	1.38E+04	745	34	54
807A-M0028-B02/M0123-B10	AVMHDK	858	3.64E-03	1.80E+04	202	1	8
807A-M0028-B02/M0123-C07	SVLFDS	706	1.25E-02	1.32E+04	945	61	63
807A-M0028-B02/M0123-C11	GVLLDI	345	9.78E-03	3.11E+04	315	29	20
*807A-M0028-B02/M0123-D01	GVLLDK	578	5.62E-03	2.65E+04	212	6	9
807A-M0028-B02/M0123-D03	SVLLDN	710	1.12E-02	1.09E+04	1030	45	68
807A-M0028-B02/M0123-D04	SVLHDY	592	1.41E-02	1.22E+04	1160	70	71
807A-M0028-B02/M0123-D06	SVLFDR	510	9.07E-03	2.13E+04	427	20	35
807A-M0028-B02/M0123-D08	SVLLDK	1013	9.26E-03	1.08E+04	862	23	60
807A-M0028-B02/M0123-E05	GGLLDY	884	1.06E-02	1.10E+04	976	37	65
807A-M0028-B02/M0123-E12	SVMFDY	646	1.13E-02	1.14E+04	1000	46	66
807A-M0028-B02/M0123-F01	SILHDY	1049	1.21E-02	7.34E+03	1660	56	72
*807A-M0028-B02/M0123-F04	GILHDY	718	4.82E-03	2.07E+04	233	5	13
807A-M0028-B02/M0123-F11	SVIFDY	522	1.26E-02	1.72E+04	739	62	53
807A-M0028-B02/M0123-F12	SILFDN	737	9.08E-03	1.45E+04	630	21	49
807A-M0028-B02/M0123-G02	AILLDY	190	1.55E-02	2.09E+04	747	73	55
807A-M0028-B02/M0123-G03	AILLDH	394	1.25E-02	1.76E+04	717	60	52
807A-M0028-B02/M0123-G12	SILFDT	628	8.17E-03	2.01E+04	409	15	34
807A-M0028-B02/M0123-H02	AVLLDY	276	1.11E-02	2.77E+04	404	42	33
807A-M0028-B02/M0123-H09	SVLPDN	685	8.78E-03	2.04E+04	434	16	36
807A-M0028-B02/M0123-H10	GILLDK	591	6.36E-03	2.85E+04	224	8	10
807A-M0028-B02/M0123-H11	SVLFDN	619	1.15E-02	1.95E+04	596	48	47
807A-M0028-B02/M0124-A01	SVLLDS	738	6.01E-03	1.75E+04	344	7	23
807A-M0028-B02/M0124-A10	SDLRAE	815	9.75E-03	1.15E+04	855	28	59
807A-M0028-B02/M0124-A11	GVLLDY	452	8.85E-03	3.47E+04	257	17	16
*807A-M0028-B02/M0124-B02	GVLHDY	649	4.40E-03	2.66E+04	166	3	5
807A-M0028-B02/M0124-B03	SVLLDR	201	1.25E-02	4.88E+04	258	59	17
807A-M0028-B02/M0124-B08	SILHDK	590	6.37E-03	3.20E+04	200	9	7
*807A-M0028-B02/M0124-B11	SILFDK	389	4.49E-03	3.82E+04	118	4	2
807A-M0028-B02/M0124-C01	SILLDH	517	1.17E-02	2.00E+04	590	49	46
807A-M0028-B02/M0124-C02	SVPIDH	243	1.24E-02	3.63E+04	344	58	24
807A-M0028-B02/M0124-C03	PVLLHF	269	8.87E-03	3.87E+04	231	19	11
807A-M0028-B02/M0124-C04	GVLLEP	509	9.99E-03	2.05E+04	494	31	41

*807A-M0028-B02/M0124-C05	GVLFDN	230	3.93E-03	7.01E+04	56	2	1
807A-M0028-B02/M0124-C06	AILLDK	272	8.08E-03	5.04E+04	162	14	4
807A-M0028-B02/M0124-D02	STLLDH	445	1.11E-02	2.07E+04	541	43	43
807A-M0028-B02/M0124-D06	SIHLDY	470	1.23E-02	1.40E+04	888	57	62
807A-M0028-B02/M0124-D08	SVTLDA	446	1.20E-02	2.23E+04	543	53	45
807A-M0028-B02/M0124-D09	SVLHDF	378	1.30E-02	2.87E+04	459	65	39
807A-M0028-B02/M0124-D10	SVLHDS	390	1.39E-02	2.22E+04	634	69	51
807A-M0028-B02/M0124-D12	GGLLDK	853	7.07E-03	2.16E+04	328	11	22
807A-M0028-B02/M0124-E02	AVLLDT	459	1.04E-02	2.74E+04	383	35	30
807A-M0028-B02/M0124-E03	AVLHDY	490	1.00E-02	2.21E+04	456	32	38
807A-M0028-B02/M0124-E04	SVLHDQ	272	1.21E-02	3.06E+04	399	54	32
807A-M0028-B02/M0124-E10	GVLLDN	493	9.68E-03	2.74E+04	357	26	26
807A-M0028-B02/M0124-F03	SVLLDH	513	9.24E-03	2.87E+04	325	22	21
807A-M0028-B02/M0124-F05	AVLHDS	211	1.19E-02	2.66E+04	451	52	37
807A-M0028-B02/M0124-G03	YVHPDY	655	1.32E-02	1.37E+04	971	68	64
807A-M0028-B02/M0124-G07	SVLHDH	516	9.81E-03	2.61E+04	380	30	29
807A-M0028-B02/M0124-G10	AVLLDN	316	1.08E-02	3.87E+04	282	38	18
807A-M0028-B02/M0125-C03	SVLLDR	473	1.18E-02	3.12E+04	385	50	31
807A-M0028-B02/M0125-D03	SVLFDY	295	1.11E-02	4.41E+04	254	40	14
807A-M0028-B02/M0125-D06	SVHLDY	161	1.12E-02	8.30E+04	136	44	3
807A-M0028-B02/M0125-D09	AVLHDS	639	1.06E-02	1.97E+04	542	36	44
807A-M0028-B02/M0125-F07	SVLLDQ	607	1.29E-02	2.06E+04	631	64	50
807A-M0028-B02/M0125-F11	SVLFDS	621	1.18E-02	2.39E+04	498	51	42
807A-M0028-B02/M0125-G02	SVLLDH	511	1.32E-02	2.21E+04	607	67	48
807A-M0028-B02/M0126-C09	AVLLDY	329	1.10E-02	3.18E+04	350	39	25
807A-M0028-B02/M0126-E03	AVLLDN	257	1.11E-02	3.86E+04	290	41	19
807A-M0028-B02/M0126-F08	SILFDY	335	9.35E-03	4.07E+04	232	24	12
807A-M0028-B02/M0126-G03	SVLHDN	627	1.28E-02	1.53E+04	846	63	58
807A-M0028-B02/M0126-G07	AVLLDH	221	1.15E-02	5.41E+04	194	47	6

* = selected clones
BC = Biacore

Table 36: Detailed biacore analysis of 807B-M0004-A03, 807B-M0009-F06, 807A-M0028-B02 and variants

Clone name	kon (1/Ms)	koff (1/s)	KD (nM)	CDR3
807B-M004-A03 / WT	1,82E+05	2.34E-02	128	SIAAAGTDY
807B-M004-A03/M0118-B09	3,12E+05	1.96E-02	63	SIAADRTDY
807B-M004-A03/M0117-G01	1,39E+05	7,72E-03	56	SIASARTDS
807B-M004-A03/M0117-D03	2,75E+04	8,36E-03	304	SIAAARTDY
807B-M004-A03/M0117-F05	1,89E+05	1.05E-02	56	SIASAGTDH
807B-M004-A03/M0118-F03	1,35E+05	1.16E-02	86	SIAASRTDY

Clone name	kon (1/Ms)	koff (1/s)	KD (nM)	CDR3
807B-M0009F06 / WT	1,31E+05	4.45E-02	340	VGMSTYAFDI
807B-M0009F06-M0128-H01	8,72E+04	7,99E-03	92	VGMSNYGFDF
807B-M0009F06-M0127-B07	1,00E+05	8,60E-03	86	VGMSTYGFDK
807B-M0009F06-M0127-D05	1,02E+05	9,39E-03	92	VGMATYGFDI
807B-M0009F06-M0127-F09	1,18E+05	1.10E-02	93	VGMYNYGFDI
807B-M0009F06-M0127-C10	1,13E+05	1.62E-02	144	VGISTYGFDL

Clone name	kon (1/Ms)	koff (1/s)	KD (nM)	CDR3
807A-M0028-B02 / WT	9,31E+04	1.58 E-02	169	SVLLDY
807A-M0028-B02/M0124-C05	2,76E+04	5,12E-03	185	GVLFDN
807A-M0028-B02/M0124-B02	6,07E+04	6,77E-03	112	GVLHDY
807A-M0028-B02/M0124-B11	5,23E+04	5,72E-03	109	SILFDK
807A-M0028-B02/M0123-F04	5,07E+04	7,56E-03	149	GILHDY
807A-M0028-B02/M0123-D01	9,25E+04	7,85E-03	85	GVLLDK

Clone name	kon (1/Ms)	koff (1/s)	KD (nM)	CDR3
807B-M0004-H03 / WT	-	-	200	EGSAGVVKGP ARYYYYYMDV
807B-M0004-H03/M0119-B05	-	-	-	EGSSGVVKGP ARYYYYYMDA
807B-M0004-H03/M0119-C05	-	-	-	ERSVAVFKAR PRHYYYYMDV
807B-M0004-H03/M0119-F04	-	-	-	EGSARVIKGP ARYYYYEMDV
807B-M0004-H03/M0119-H09	-	-	-	DGSARVVKGP RRYYYYYIDV
807B-M0004-H03/M0120-B05	-	-	-	EGSARVVKGP ARYFYYYMDL

Table 37: Immunohistochemistry of clones selected from Biacore screening

Clone 807B-M0004-A03	IHC
Original clone	+/-
807B-M0004-A03/M0117-D03	+
807B-M0004-A03/M0117-F05	+
807B-M0004-A03/M0117-G01	+/-
807B-M0004-A03/M0118-B09	+(+)
807B-M0004-A03/M0118-F03	+/-
Clone 807B-M0004-H03	
Original clone	+/-
807B-M0004-H03/M0119-B05	++(+)
807B-M0004-H03/M0119-C05	++(+)
807B-M0004-H03/M0119-F04	++(+)
807B-M0004-H03/M0119-H09	++(+)
807B-M0004-H03/M0120-B05	++
Clone 807A-M0028-B02	
Original clone	++
807B-M0028-B02/M0123-D01	++
807B-M0028-B02/M0123-F04	++
807B-M0028-B02/M0124-B02	++
807B-M0028-B02/M0124-B11	++
807B-M0028-B02/M0124-C05	++
Clone 807B-M0009-F06	
Original clone	+/-
807B-M0009-F06/M0127-B07	+/-
807B-M0009-F06/M0127-C10	+
807B-M0009-F06/M0127-D05	-
807B-M0009-F06/M0127-F09	+/-
807B-M0009-F06/M0128-H01	+

Table 38: Affinity matured clones of 807A-M0028-B02

Initial Name	HV-CDR3	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	LV-WholeAA	HV-WholeAA
807A-M0028-B02/M0167-E01	SEQ ID NO: 207	SEQ ID NO: 211	SEQ ID NO: 240	SEQ ID NO: 262	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 287	SEQ ID NO: 316
807A-M0028-B02/M0167-E07	SEQ ID NO: 208	SEQ ID NO: 212	SEQ ID NO: 241	SEQ ID NO: 263	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 288	SEQ ID NO: 317
807A-M0028-B02/M0167-F07	SEQ ID NO: 209	SEQ ID NO: 213	SEQ ID NO: 34	SEQ ID NO: 35	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 289	SEQ ID NO: 318
807A-M0028-B02/M0167-F09	SEQ ID NO: 207	SEQ ID NO: 214	SEQ ID NO: 243	SEQ ID NO: 265	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 290	SEQ ID NO: 316
807A-M0028-B02/M0168-B11	SEQ ID NO: 209	SEQ ID NO: 215	SEQ ID NO: 244	SEQ ID NO: 266	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 291	SEQ ID NO: 318
807A-M0028-B02/M0168-C08	SEQ ID NO: 209	SEQ ID NO: 216	SEQ ID NO: 245	SEQ ID NO: 267	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 292	SEQ ID NO: 318
807A-M0028-B02/M0168-D10	SEQ ID NO: 210	SEQ ID NO: 33	SEQ ID NO: 34	SEQ ID NO: 35	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 43	SEQ ID NO: 319
807A-M0028-B02/M0169-D08	SEQ ID NO: 208	SEQ ID NO: 218	SEQ ID NO: 34	SEQ ID NO: 268	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 294	SEQ ID NO: 317
807A-M0028-B02/M0169-F03	SEQ ID NO: 209	SEQ ID NO: 219	SEQ ID NO: 247	SEQ ID NO: 269	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 295	SEQ ID NO: 318
807A-M0028-B02/M0169-H04	SEQ ID NO: 208	SEQ ID NO: 220	SEQ ID NO: 248	SEQ ID NO: 270	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 296	SEQ ID NO: 317
807A-M0028-B02/M0169-H05	SEQ ID NO: 207	SEQ ID NO: 221	SEQ ID NO: 249	SEQ ID NO: 271	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 297	SEQ ID NO: 316
807A-M0028-B02/M0170-H08	SEQ ID NO: 207	SEQ ID NO: 222	SEQ ID NO: 250	SEQ ID NO: 272	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 298	SEQ ID NO: 316
807A-M0028-B02/M0171-A08	SEQ ID NO: 210	SEQ ID NO: 223	SEQ ID NO: 240	SEQ ID NO: 273	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 299	SEQ ID NO: 319
807A-M0028-B02/M0171-A09	SEQ ID NO: 209	SEQ ID NO: 224	SEQ ID NO: 251	SEQ ID NO: 274	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 300	SEQ ID NO: 318
807A-M0028-B02/M0171-A10	SEQ ID NO: 207	SEQ ID NO: 33	SEQ ID NO: 34	SEQ ID NO: 35	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 43	SEQ ID NO: 316
807A-M0028-B02/M0171-C12	SEQ ID NO: 207	SEQ ID NO: 226	SEQ ID NO: 252	SEQ ID NO: 275	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 302	SEQ ID NO: 316
807A-M0028-B02/M0171-E03	SEQ ID NO: 209	SEQ ID NO: 218	SEQ ID NO: 34	SEQ ID NO: 268	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 294	SEQ ID NO: 318
807A-M0028-B02/M0171-G02	SEQ ID NO: 208	SEQ ID NO: 228	SEQ ID NO: 253	SEQ ID NO: 276	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 304	SEQ ID NO: 317
807A-M0028-B02/M0171-G09	SEQ ID NO: 209	SEQ ID NO: 229	SEQ ID NO: 254	SEQ ID NO: 277	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 305	SEQ ID NO: 318
807A-M0028-B02/M0172-A05	SEQ ID NO: 209	SEQ ID NO: 230	SEQ ID NO: 255	SEQ ID NO: 278	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 306	SEQ ID NO: 318
807A-M0028-B02/M0172-A08	SEQ ID NO: 208	SEQ ID NO: 231	SEQ ID NO: 256	SEQ ID NO: 279	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 307	SEQ ID NO: 317
807A-M0028-B02/M0172-B09	SEQ ID NO: 209	SEQ ID NO: 232	SEQ ID NO: 257	SEQ ID NO: 280	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 308	SEQ ID NO: 318
807A-M0028-B02/M0172-D05	SEQ ID NO: 209	SEQ ID NO: 233	SEQ ID NO: 257	SEQ ID NO: 281	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 309	SEQ ID NO: 318
807A-M0028-B02/M0172-D09	SEQ ID NO: 207	SEQ ID NO: 234	SEQ ID NO: 258	SEQ ID NO: 282	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 310	SEQ ID NO: 316
807A-M0028-B02/M0172-E06	SEQ ID NO: 209	SEQ ID NO: 235	SEQ ID NO: 251	SEQ ID NO: 283	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 311	SEQ ID NO: 318
807A-M0028-B02/M0172-F02	SEQ ID NO: 207	SEQ ID NO: 236	SEQ ID NO: 259	SEQ ID NO: 284	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 312	SEQ ID NO: 316
807A-M0028-B02/M0172-F07	SEQ ID NO: 210	SEQ ID NO: 213	SEQ ID NO: 34	SEQ ID NO: 35	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 289	SEQ ID NO: 319
807A-M0028-B02/M0172-F12	SEQ ID NO: 209	SEQ ID NO: 238	SEQ ID NO: 260	SEQ ID NO: 285	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 314	SEQ ID NO: 318
807A-M0028-B02/M0172-G08	SEQ ID NO: 209	SEQ ID NO: 239	SEQ ID NO: 261	SEQ ID NO: 286	SEQ ID NO: 24	SEQ ID NO: 25	SEQ ID NO: 315	SEQ ID NO: 318

Table 39: Affinity matured clones of 807B-M0004-A03

Initial Name	HV-CDR3_1	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	LV-WholeAA	HV-WholeAA
807B-M0004-A03/M0148-E05	SEQ ID NO: 320	SEQ ID NO: 93	SEQ ID NO: 333	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 345	SEQ ID NO: 369
807B-M0004-A03/M0148-E08	SEQ ID NO: 320	SEQ ID NO: 325	SEQ ID NO: 333	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 346	SEQ ID NO: 369
807B-M0004-A03/M0149-D04	SEQ ID NO: 322	SEQ ID NO: 326	SEQ ID NO: 334	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 347	SEQ ID NO: 370
807B-M0004-A03/M0149-F02	SEQ ID NO: 322	SEQ ID NO: 93	SEQ ID NO: 333	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 348	SEQ ID NO: 370
807B-M0004-A03/M0149-G11	SEQ ID NO: 323	SEQ ID NO: 93	SEQ ID NO: 333	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 348	SEQ ID NO: 371
807B-M0004-A03/M0149-H07	SEQ ID NO: 322	SEQ ID NO: 326	SEQ ID NO: 334	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 350	SEQ ID NO: 370
807B-M0004-A03/M0149-H09	SEQ ID NO: 320	SEQ ID NO: 327	SEQ ID NO: 333	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 351	SEQ ID NO: 369
807B-M0004-A03/M0150-A04	SEQ ID NO: 320	SEQ ID NO: 93	SEQ ID NO: 333	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 352	SEQ ID NO: 369
807B-M0004-A03/M0150-A07	SEQ ID NO: 321	SEQ ID NO: 93	SEQ ID NO: 333	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 348	SEQ ID NO: 372
807B-M0004-A03/M0150-A12	SEQ ID NO: 320	SEQ ID NO: 93	SEQ ID NO: 333	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 354	SEQ ID NO: 369
807B-M0004-A03/M0150-D12	SEQ ID NO: 320	SEQ ID NO: 325	SEQ ID NO: 333	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 355	SEQ ID NO: 369
807B-M0004-A03/M0150-E01	SEQ ID NO: 320	SEQ ID NO: 93	SEQ ID NO: 335	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 356	SEQ ID NO: 369
807B-M0004-A03/M0150-E03	SEQ ID NO: 320	SEQ ID NO: 325	SEQ ID NO: 333	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 357	SEQ ID NO: 369
807B-M0004-A03/M0150-E04	SEQ ID NO: 320	SEQ ID NO: 327	SEQ ID NO: 333	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 358	SEQ ID NO: 369
807B-M0004-A03/M0150-E12	SEQ ID NO: 320	SEQ ID NO: 328	SEQ ID NO: 336	SEQ ID NO: 342	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 359	SEQ ID NO: 369
807B-M0004-A03/M0150-G01	SEQ ID NO: 320	SEQ ID NO: 329	SEQ ID NO: 337	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 360	SEQ ID NO: 369
807B-M0004-A03/M0151-A06	SEQ ID NO: 320	SEQ ID NO: 330	SEQ ID NO: 338	SEQ ID NO: 343	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 361	SEQ ID NO: 369
807B-M0004-A03/M0151-B12	SEQ ID NO: 320	SEQ ID NO: 325	SEQ ID NO: 333	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 362	SEQ ID NO: 369
807B-M0004-A03/M0151-C05	SEQ ID NO: 320	SEQ ID NO: 328	SEQ ID NO: 336	SEQ ID NO: 342	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 363	SEQ ID NO: 369
807B-M0004-A03/M0151-D09	SEQ ID NO: 322	SEQ ID NO: 325	SEQ ID NO: 333	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 362	SEQ ID NO: 370
807B-M0004-A03/M0151-F12	SEQ ID NO: 320	SEQ ID NO: 93	SEQ ID NO: 333	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 348	SEQ ID NO: 369
807B-M0004-A03/M0151-H05	SEQ ID NO: 320	SEQ ID NO: 331	SEQ ID NO: 339	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 366	SEQ ID NO: 369
807B-M0004-A03/M0153-D03	SEQ ID NO: 320	SEQ ID NO: 93	SEQ ID NO: 94	SEQ ID NO: 95	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 151	SEQ ID NO: 369
807B-M0004-A03/M0153-F07	SEQ ID NO: 320	SEQ ID NO: 327	SEQ ID NO: 333	SEQ ID NO: 341	SEQ ID NO: 48	SEQ ID NO: 49	SEQ ID NO: 368	SEQ ID NO: 369

Table 40: Affinity matured clones of 807B-M0004-H03

Initial Name	HV-CDR3	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	LV-WholeAA	HV-WholeAA
807B-M0004-H03/M0154-C07	SEQ ID NO: 373	SEQ ID NO: 388	SEQ ID NO: 381	SEQ ID NO: 377	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 400	SEQ ID NO: 397
807B-M0004-H03/M0154-D08	SEQ ID NO: 373	SEQ ID NO: 389	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 401	SEQ ID NO: 397
807B-M0004-H03/M0154-G05	SEQ ID NO: 373	SEQ ID NO: 390	SEQ ID NO: 383	SEQ ID NO: 379	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 402	SEQ ID NO: 397
807B-M0004-H03/M0154-G08	SEQ ID NO: 373	SEQ ID NO: 389	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 403	SEQ ID NO: 397
807B-M0004-H03/M0154-G11	SEQ ID NO: 374	SEQ ID NO: 389	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 404	SEQ ID NO: 217
807B-M0004-H03/M0154-H03	SEQ ID NO: 375	SEQ ID NO: 389	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 405	SEQ ID NO: 398
807B-M0004-H03/M0154-H06	SEQ ID NO: 373	SEQ ID NO: 391	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 406	SEQ ID NO: 397
807B-M0004-H03/M0155-C08	SEQ ID NO: 373	SEQ ID NO: 389	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 407	SEQ ID NO: 397
807B-M0004-H03/M0155-E10	SEQ ID NO: 376	SEQ ID NO: 392	SEQ ID NO: 384	SEQ ID NO: 380	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 408	SEQ ID NO: 399
807B-M0004-H03/M0155-F08	SEQ ID NO: 373	SEQ ID NO: 389	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 409	SEQ ID NO: 397
807B-M0004-H03/M0155-H06	SEQ ID NO: 373	SEQ ID NO: 389	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 410	SEQ ID NO: 397
807B-M0004-H03/M0155-H08	SEQ ID NO: 376	SEQ ID NO: 389	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 411	SEQ ID NO: 399
807B-M0004-H03/M0156-D01	SEQ ID NO: 373	SEQ ID NO: 389	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 412	SEQ ID NO: 397
807B-M0004-H03/M0156-G08	SEQ ID NO: 373	SEQ ID NO: 389	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 413	SEQ ID NO: 397
807B-M0004-H03/M0157-A08	SEQ ID NO: 373	SEQ ID NO: 389	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 414	SEQ ID NO: 397
807B-M0004-H03/M0157-D10	SEQ ID NO: 376	SEQ ID NO: 389	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 415	SEQ ID NO: 399
807B-M0004-H03/M0157-G08	SEQ ID NO: 373	SEQ ID NO: 389	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 416	SEQ ID NO: 397
807B-M0004-H03/M0157-G11	SEQ ID NO: 375	SEQ ID NO: 393	SEQ ID NO: 385	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 417	SEQ ID NO: 398
807B-M0004-H03/M0159-A09	SEQ ID NO: 373	SEQ ID NO: 394	SEQ ID NO: 386	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 418	SEQ ID NO: 397
807B-M0004-H03/M0159-H03	SEQ ID NO: 373	SEQ ID NO: 389	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 419	SEQ ID NO: 397
807B-M0004-H03/M0159-H07	SEQ ID NO: 373	SEQ ID NO: 395	SEQ ID NO: 387	SEQ ID NO: 380	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 420	SEQ ID NO: 397
807B-M0004-H03/M0157-F04	SEQ ID NO: 68	SEQ ID NO: 396	SEQ ID NO: 382	SEQ ID NO: 378	SEQ ID NO: 66	SEQ ID NO: 67	SEQ ID NO: 421	SEQ ID NO: 142

Table 41: Affinity matured clones of 807B-M0009-F06

Initial Name	HV-CDR3_1	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	LV-WholeAA	HV-WholeAA
807B-M0009-F06/M0173-F07	SEQ ID NO: 485	SEQ ID NO: 117	SEQ ID NO: 118	SEQ ID NO: 119	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 508	SEQ ID NO: 500
807B-M0009-F06/M0174-B01	SEQ ID NO: 486	SEQ ID NO: 117	SEQ ID NO: 118	SEQ ID NO: 119	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 508	SEQ ID NO: 490
807B-M0009-F06/M0174-B06	SEQ ID NO: 487	SEQ ID NO: 117	SEQ ID NO: 118	SEQ ID NO: 119	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 508	SEQ ID NO: 491
807B-M0009-F06/M0174-B08	SEQ ID NO: 488	SEQ ID NO: 117	SEQ ID NO: 118	SEQ ID NO: 119	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 508	SEQ ID NO: 509
807B-M0009-F06/M0175-A07	SEQ ID NO: 488	SEQ ID NO: 501	SEQ ID NO: 118	SEQ ID NO: 101	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 227	SEQ ID NO: 509
807B-M0009-F06/M0175-B01	SEQ ID NO: 485	SEQ ID NO: 502	SEQ ID NO: 497	SEQ ID NO: 101	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 237	SEQ ID NO: 500
807B-M0009-F06/M0175-B11	SEQ ID NO: 485	SEQ ID NO: 503	SEQ ID NO: 498	SEQ ID NO: 119	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 242	SEQ ID NO: 500
807B-M0009-F06/M0175-C07	SEQ ID NO: 488	SEQ ID NO: 501	SEQ ID NO: 118	SEQ ID NO: 101	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 246	SEQ ID NO: 509
807B-M0009-F06/M0175-D04	SEQ ID NO: 485	SEQ ID NO: 503	SEQ ID NO: 498	SEQ ID NO: 119	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 264	SEQ ID NO: 500
807B-M0009-F06/M0175-E04	SEQ ID NO: 485	SEQ ID NO: 503	SEQ ID NO: 118	SEQ ID NO: 492	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 293	SEQ ID NO: 500
807B-M0009-F06/M0175-E06	SEQ ID NO: 486	SEQ ID NO: 503	SEQ ID NO: 118	SEQ ID NO: 119	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 301	SEQ ID NO: 490
807B-M0009-F06/M0176-A06	SEQ ID NO: 485	SEQ ID NO: 504	SEQ ID NO: 118	SEQ ID NO: 101	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 303	SEQ ID NO: 500
807B-M0009-F06/M0176-C04	SEQ ID NO: 485	SEQ ID NO: 505	SEQ ID NO: 499	SEQ ID NO: 493	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 313	SEQ ID NO: 500
807B-M0009-F06/M0176-G02	SEQ ID NO: 485	SEQ ID NO: 225	SEQ ID NO: 118	SEQ ID NO: 101	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 324	SEQ ID NO: 500
807B-M0009-F06/M0177-E01	SEQ ID NO: 489	SEQ ID NO: 117	SEQ ID NO: 118	SEQ ID NO: 119	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 332	SEQ ID NO: 510
807B-M0009-F06/M0177-E05	SEQ ID NO: 486	SEQ ID NO: 117	SEQ ID NO: 118	SEQ ID NO: 119	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 340	SEQ ID NO: 490
807B-M0009-F06/M0177-E09	SEQ ID NO: 488	SEQ ID NO: 117	SEQ ID NO: 118	SEQ ID NO: 119	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 344	SEQ ID NO: 509
807B-M0009-F06/M0177-F09	SEQ ID NO: 488	SEQ ID NO: 503	SEQ ID NO: 118	SEQ ID NO: 494	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 349	SEQ ID NO: 509
807B-M0009-F06/M0177-H02	SEQ ID NO: 488	SEQ ID NO: 503	SEQ ID NO: 118	SEQ ID NO: 119	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 353	SEQ ID NO: 509
807B-M0009-F06/M0177-H05	SEQ ID NO: 489	SEQ ID NO: 117	SEQ ID NO: 118	SEQ ID NO: 119	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 508	SEQ ID NO: 510
807B-M0009-F06/M0177-H06	SEQ ID NO: 488	SEQ ID NO: 505	SEQ ID NO: 499	SEQ ID NO: 493	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 364	SEQ ID NO: 509
807B-M0009-F06/M0177-H07	SEQ ID NO: 488	SEQ ID NO: 506	SEQ ID NO: 118	SEQ ID NO: 495	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 365	SEQ ID NO: 509
807B-M0009-F06/M0178-A08	SEQ ID NO: 488	SEQ ID NO: 503	SEQ ID NO: 118	SEQ ID NO: 496	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 367	SEQ ID NO: 509
807B-M0009-F06/M0178-E02	SEQ ID NO: 486	SEQ ID NO: 507	SEQ ID NO: 118	SEQ ID NO: 494	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 439	SEQ ID NO: 490
807B-M0009-F06/M0178-H09	SEQ ID NO: 488	SEQ ID NO: 505	SEQ ID NO: 499	SEQ ID NO: 493	SEQ ID NO: 72	SEQ ID NO: 73	SEQ ID NO: 442	SEQ ID NO: 509

Table 42: Affinity matured clones of 807B-M0079-D10

Initial Name	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3	LV-WholeAA	HV-WholeAA
807B-M0079-D10/M0160-F02	SEQ ID NO: 422	SEQ ID NO: 434	SEQ ID NO: 444	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 455	SEQ ID NO: 146
807B-M0079-D10/M0160-F12	SEQ ID NO: 423	SEQ ID NO: 435	SEQ ID NO: 445	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 456	SEQ ID NO: 146
807B-M0079-D10/M0161-B04	SEQ ID NO: 424	SEQ ID NO: 434	SEQ ID NO: 446	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 457	SEQ ID NO: 146
807B-M0079-D10/M0161-G03	SEQ ID NO: 423	SEQ ID NO: 435	SEQ ID NO: 445	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 458	SEQ ID NO: 146
807B-M0079-D10/M0162-A11	SEQ ID NO: 425	SEQ ID NO: 435	SEQ ID NO: 447	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 459	SEQ ID NO: 146
807B-M0079-D10/M0162-D11	SEQ ID NO: 424	SEQ ID NO: 434	SEQ ID NO: 445	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 460	SEQ ID NO: 146
807B-M0079-D10/M0162-F04	SEQ ID NO: 426	SEQ ID NO: 436	SEQ ID NO: 448	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 461	SEQ ID NO: 146
807B-M0079-D10/M0164-B02	SEQ ID NO: 423	SEQ ID NO: 435	SEQ ID NO: 445	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 462	SEQ ID NO: 146
807B-M0079-D10/M0164-B03	SEQ ID NO: 425	SEQ ID NO: 435	SEQ ID NO: 447	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 463	SEQ ID NO: 146
807B-M0079-D10/M0164-C08	SEQ ID NO: 425	SEQ ID NO: 435	SEQ ID NO: 447	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 464	SEQ ID NO: 146
807B-M0079-D10/M0164-C09	SEQ ID NO: 427	SEQ ID NO: 437	SEQ ID NO: 449	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 465	SEQ ID NO: 146
807B-M0079-D10/M0164-C10	SEQ ID NO: 428	SEQ ID NO: 438	SEQ ID NO: 450	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 466	SEQ ID NO: 146
807B-M0079-D10/M0164-E05	SEQ ID NO: 424	SEQ ID NO: 434	SEQ ID NO: 446	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 467	SEQ ID NO: 146
807B-M0079-D10/M0165-A02	SEQ ID NO: 429	SEQ ID NO: 440	SEQ ID NO: 451	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 468	SEQ ID NO: 146
807B-M0079-D10/M0165-A06	SEQ ID NO: 430	SEQ ID NO: 441	SEQ ID NO: 452	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 469	SEQ ID NO: 146
807B-M0079-D10/M0165-B07	SEQ ID NO: 423	SEQ ID NO: 435	SEQ ID NO: 445	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 470	SEQ ID NO: 146
807B-M0079-D10/M0165-B08	SEQ ID NO: 428	SEQ ID NO: 438	SEQ ID NO: 450	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 471	SEQ ID NO: 146
807B-M0079-D10/M0165-D03	SEQ ID NO: 431	SEQ ID NO: 434	SEQ ID NO: 445	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 472	SEQ ID NO: 146
807B-M0079-D10/M0165-D05	SEQ ID NO: 428	SEQ ID NO: 438	SEQ ID NO: 450	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 473	SEQ ID NO: 146
807B-M0079-D10/M0165-D12	SEQ ID NO: 428	SEQ ID NO: 438	SEQ ID NO: 450	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 474	SEQ ID NO: 146
807B-M0079-D10/M0165-E01	SEQ ID NO: 426	SEQ ID NO: 436	SEQ ID NO: 448	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 475	SEQ ID NO: 146
807B-M0079-D10/M0166-A08	SEQ ID NO: 429	SEQ ID NO: 440	SEQ ID NO: 451	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 476	SEQ ID NO: 146
807B-M0079-D10/M0166-A09	SEQ ID NO: 424	SEQ ID NO: 434	SEQ ID NO: 446	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 477	SEQ ID NO: 146
807B-M0079-D10/M0166-B08	SEQ ID NO: 423	SEQ ID NO: 435	SEQ ID NO: 445	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 478	SEQ ID NO: 146
807B-M0079-D10/M0166-C08	SEQ ID NO: 430	SEQ ID NO: 441	SEQ ID NO: 452	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 479	SEQ ID NO: 146
807B-M0079-D10/M0166-D03	SEQ ID NO: 432	SEQ ID NO: 443	SEQ ID NO: 454	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 480	SEQ ID NO: 146
807B-M0079-D10/M0166-F03	SEQ ID NO: 423	SEQ ID NO: 435	SEQ ID NO: 445	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 481	SEQ ID NO: 146
807B-M0079-D10/M0166-F04	SEQ ID NO: 425	SEQ ID NO: 435	SEQ ID NO: 447	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 482	SEQ ID NO: 146
807B-M0079-D10/M0166-F07	SEQ ID NO: 433	SEQ ID NO: 434	SEQ ID NO: 445	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 483	SEQ ID NO: 146
807B-M0079-D10/M0166-F08	SEQ ID NO: 424	SEQ ID NO: 434	SEQ ID NO: 446	SEQ ID NO: 78	SEQ ID NO:453	SEQ ID NO: 80	SEQ ID NO: 484	SEQ ID NO: 146

Table 43: Light chain sequences of antibodies selected by VH-CDR3 spiking and light chain shuffling

Initial Name	LV-FR1	LV-CDR1	LV-FR2	LV-CDR2	LV-FR3	LV-CDR3	LV-FR4
807B-M0004-A03 /M0149-D04	QSVLTQSPSASGTPGQRVTISC	SGSNSNVGTKTVN	WYQVLPGTAPKLLIY	SNTQRPS	GVPDRFSGSKSGTSASLAISGLQSEDEADYYC	AAWDDSLNGPV	FGGGTRVTVL
807B-M0004-A03 /M0149-F02	QSALTQPPSASGTPGQRVTISC	SGSSSNIGSNTVN	WYQQLPGTAPKLLIY	SNNQRPS	GVPDRFSGSKSGTSASLAISGLQSEDEADYYC	AAWDDSLNGPV	FGGGTKLTVL
807B-M0004-A03 /M0150-A07	QSALTQPPSASGTPGQRVTISC	SGSSSNIGSNTVN	WYQQLPGTAPKLLIY	SNNQRPS	GVPDRFSGSKSGTSASLAISGLQSEDEADYYC	AAWDDSLNGPV	FGGGTKLTVL
807B-M0004-A03 /M0150-E03	QYELTQPPSASGTPGQRVTISC	SGSSSNIGINTVN	WYQQLPGTAPKLLIY	SNNQRPS	GVPDRFSGSKSGTSASLAISGLQSEDEADYYC	AAWDDSLNGPV	FGGGTKLTVL
807B-M0004-A03 /M0151-D09	QSALTQPPSASGTPGQRVTISC	SGSSSNIGINTVN	WYQQLPGTAPKLLIY	SNNQRPS	GVPDRFSGSKSGTSASLAISGLQSEDEADYYC	AAWDDSLNGPV	FGGGTKLTVL
807A-M0028-B02 /M0169-F03	QDIQMTQSPSSLSASVGDRVTITC	QASQDISNYLN	WYQQKPGKAPQRLIY	GASTVQS	GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC	QQYKTYPFT	FGQGTRLDIK
807A-M0028-B02 /M0171-E03	QDIQMTQSPSSLSASVGDRVTITC	RTSQDIGNHLA	WYQQKPGKAPQRLIR	EASILQS	GVPSTFSGSGSGTEFTLTISSLQPEDFASYYC	QQYDAFPFT	FGQGTKLEIK
807A-M0028-B02 /M0171-G02	QDIQMTQSPSSLSASVGDTVTITC	RASQGITNYLA	WFQQKPGKAPKSLM	YGAYKLQY	GVPTKFSGSGSGTDFTLTIRSLQPEDFATYYC	LQYQTYPFT	FGPGTKVDLK
807A-M0028-B02 /M0172-F07	QDIQMTQSPSSLSASVGDRVTITC	RTSQGIRNHLG	WFQQKPGKAPQRLIR	EASILQS	GVPSTFSGSGSGTEFTLTISSLQPEDFATYYC	LQYDSFPYT	FGQGTKLEIK
807B-M0004-H03 /M0154-H06	QDIQMTQSPSSLSASVGDRVTITC	RASRGVSTSLN	WYQIKPEKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLAITSLQPEDFATYYC	QQSYSTPRT	FGPGTKVEIK
807B-M0004-H03 /M0159-A09	QDIQMTQSPSSLSASVGDRVTITC	RASQSIRSYLN	WFQQKPGKAPKLLIY	AASTLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTPRT	FGQGTKLEIK
807A-M0028-B02 /M0168-D10	QDIQMTQSPSSLSASVGDRVTITC	RTSQDIRNHLG	WFQQKPGKAPQRLIR	EASILQS	GVPSTFYGSGYGREFTLTISSLQPEDFATYYC	LQYDSFPYT	FGQGTKLEIK
807B-M0004-A03 /M0149-G11	QSALTQPPSASGTPGQRVTISC	SGSSSNIGSNTVN	WYQQLPGTAPKLLIY	SNNQRPS	GVPDRFSGSKSGTSASLAISGLQSEDEADYYC	AAWDDSLNGPV	FGGGTKLTVL

Table 44: Heavy chain sequences of antibodies selected by VH-CDR3 spiking and light chain shuffling

Initial Name	HV-FR1	HV-CDR1	HV-FR2	HV-CDR2	HV-FR3	HV-CDR3	HV-FR4
807B-M0004-A03/M0149-D04	EVQLLESGGGLVQPGGSLRL SCAASGFTFS	RYLMM	WVRQAPGKGLEWVS	VISPSGGRTWYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTA VYYCVR	SIASAGTDH	WGQGTLVTVSS
807B-M0004-A03/M0149-F02	EVQLLESGGGLVQPGGSLRL SCAASGFTFS	RYLMM	WVRQAPGKGLEWVS	VISPSGGRTWYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTA VYYCVR	SIASAGTDH	WGQGTLVTVSS
807B-M0004-A03/M0150-A07	EVQLLESGGGLVQPGGSLRL SCAASGFTFS	RYLMM	WVRQAPGKGLEWVS	VISPSGGRTWYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTA VYYCVR	SIAASRTDY	WGQGTLVTVSS
807B-M0004-A03/M0150-E03	EVQLLESGGGLVQPGGSLRL SCAASGFTFS	RYLMM	WVRQAPGKGLEWVS	VISPSGGRTWYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTA VYYCVR	SIAADRTDY	WGQGTLVTVSS
807B-M0004-A03/M0151-D09	EVQLLESGGGLVQPGGSLRL SCAASGFTFS	RYLMM	WVRQAPGKGLEWVS	VISPSGGRTWYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTA VYYCVR	SIASAGTDH	WGQGTLVTVSS
807A-M0028-B02/M0169-F03	EVQLLESGGGLVQPGGSLRL SCAASGFTFS	MYMMD	WVRQAPGKGLEWVS	SIWPSGGQTWYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR	GVLLDK	WGQGTLVTVSS
807A-M0028-B02/M0171-E03	EVQLLESGGGLVQPGGSLRL SCAASGFTFS	MYMMD	WVRQAPGKGLEWVS	SIWPSGGQTWYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR	GVLLDK	WGQGTLVTVSS
807A-M0028-B02/M0171-G02	EVQLLESGGGLVQPGGSLRL SCAASGFTFS	MYMMD	WVRQAPGKGLEWVS	SIWPSGGQTWYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR	GILHDY	WGQGTLVTVSS
807A-M0028-B02/M0172-F07	EVQLLESGGGLVQPGGSLRL SCAASGFTFS	MYMMD	WVRQAPGKGLEWVS	SIWPSGGQTWYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR	GVLFDN	WGQGTLVTVSS
807B-M0004-H03/M0154-H06	EVQLLESGGGLVQPGGSLRL SCAASGFTFS	SYPMV	WVRQAPGKGLEWVS	GIWSSGGLTYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR	ERSVAVFKARPRHYYYY MDV	WGKGTTVTVSS
807B-M0004-H03/M0159-A09	EVQLLESGGGLVQPGGSLRL SCAASGFTFS	SYPMV	WVRQAPGKGLEWVS	GIWSSGGLTYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR	ERSVAVFKARPRHYYYY MDV	WGKGTTVTVSS
807A-M0028-B02/M0168-D10	EVQLLESGGGLVQPGGSLRL SCAASGFTFS	MYMMD	WVRQAPGKGLEWVS	SIWPSGGQTWYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTA VYYCAR	GVLFDN	WGQGTLVTVSS
807B-M0004-A03/M0149-G11	EVQLLESGGGLVQPGGSLRL SCAASGFTFS	RYLMM	WVRQAPGKGLEWVS	VISPSGGRTWYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTA VYYCVR	SIASARTDS	WGQGTLVTVSS